
RESULTS AND DISCUSSION

Industrial effluents are undesirable by-products of economic development and technological innovation. When improperly disposed off, they endanger human health and the environment (Gomez *et al.*, 2008). Unfortunately, the accurate amount of dyes produced in the world is not known. It is estimated to be greater than 10,000 tons per year. Exact data on the quantity of dyes discharged into the environment are also not available. Because of their commercial importance, the impact of dyes that are released into the environment have been widely studied (Guaratini and Zanoni, 2000). It has been established that some chemicals are not totally removed because the conventional technology of treatment used in wastewater treatment plants seems to be inadequate for the complete removal of these compounds. In many developed countries, toxicity tests on industrial effluents are necessary to ensure that such discharges will not have adverse effects on the environment. A wide range of methods have been urbanized for the removal of synthetic dyes from waters and wastewaters to decrease their impact on the environment. The technologies involve absorption of inorganic or organic materials, decolorization by photocatalysis and by oxidation processes, microbiological or enzymatic decomposition, etc (Hao *et al.*, 2000).

But in developing countries, many industries are operated as small and medium level industries. The small scale industries discharge the effluent directly into the environment devoid of any pretreatment. The ecological and toxicological problems resulting from the discharge of wastewaters from these industries into the drainage and a need for the development of low cost, easily available method that can remove the harmful chemicals present in industrial effluent are the most vital challenges.

Hence, in the present study, the impact of the biofertilizer on the silk dyeing effluent and its effect on the selected GLVs were assessed. The results of the study entitled “Biofertilizer mediated remediation of silk dyeing industrial effluent and its effect on selected green leafy vegetables” are discussed as follows.

Phase 1

4.1 Growth studies of selected green leafy vegetables grown in fresh water

- 4.1.1 Biometric parameters of the five selected green leafy vegetables
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Phase 2

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Phase 4

4.4. Identification of the functional groups and compounds in the selected GLV plants using spectroscopic and chromatographic techniques

- 4.4.1. Analysis of the methanolic extracts of the selected GLVs grown in fresh water in bionano UV visible spectrophotometer
- 4.4.2 FT-IR analysis of the selected GLVs grown in different treatments and selected dyes in silk dyeing effluent
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Phase 1

4.1 Growth studies of selected green leafy vegetables grown in fresh water

Five green leafy vegetables namely mustard (*Brassica juncea*), fenugreek (*Trigonella foenum*), sirukeerai (*Amaranthus polygonoides*), araikeerai (*Amaranthus tristis*) and agati (*Sesbania grandiflora*) were grown in fresh water under normal conditions which act as the control.

Plate 2

Growth of the GLVs in fresh water



Brassica juncea (Mustard)



Trigonella foenum (Fenugreek)



Amaranthus polygonoides (Sirukeerai)



Amaranthus tristis (Araikeerai)



Sesbania grandiflora (Agati)

The growth of the selected GLVs in fresh water is depicted in Plate 2. The GLVs were post harvested on the 45th day without any damage and were analysed for parameters like biometric, phytochemicals, proximate principles, mineral contents, antioxidants, pigments and histological studies of the leaf, the stem and the root. The physico-chemical analysis of the control soil was also carried out.

4.1.1 Biometric parameters of the five selected green leafy vegetables

The plant growth analysis refers to the set of quantitative methods that depict and interpret the performance of whole plant systems grown under natural, seminatural with controlled conditions (Hunt, 2003).

Table 2

Biometric parameters of the five selected GLV plants grown in fresh water

Parameters	<i>Brassica juncea</i> (BJN)	<i>Trigonella foenum</i> (TFN)	<i>Amaranthus polygonoides</i> (APN)	<i>Amaranthus tristis</i> (ATN)	<i>Sesbania grandiflora</i> (SGN)
First cotyledon	Within 2 days			Within 3 days	
Seed germination (%)	90.0±3.7	93.3±8.7	94.2±3.6	85.7±5.7	80.0±0.8
Seedling length (cm)	53±5.6	45±0.4	55±0.8	50±7.0	97±1.6
Number of Leaves plant ⁻¹	101±5.4	90±5.1	79±1.2	67±2.4	200±4.8
Surface area of leaves (sq/cm)	22±0.2	4±0.1	9±0.2	7±0.3	11±0.2
Root length (cm)	10±0.5	14±0.8	26±2.1	15±4.0	23±0.1
Shoot length (cm)	43±2.9	31±0.9	29±0.7	35±3.5	74±2.6
Root shoot ratio	1.30	2.00	1.16	0.66	2.10
Root mass (g)	4.5±0.3	2.7±0.2	3.5±0.4	3.0±0.2	7.0±0.1
Dry matter (%)	72.2±0.8	55.1±1.4	77.2±0.8	71.4±0.8	86.5±0.4
Number of Seed bunch plant ⁻¹	28±0.5	5±1.6	15±0.4	20±0.5	-
Distance between the nodes (cm)	2.1±0.08	2.3±0.80	3.2±0.60	3.5±0.30	4.0±0.80

Values are the mean of three replicates ± SD

Note- *S. grandiflora* did not produce seeds within the experimental period

BJN: *Brassica juncea*, TFN: *Trigonella foenum*, APN: *Amaranthus polygonoides*, ATN: *Amaranthus tristis*, SGN: *Sesbania grandiflora* were grown in fresh water.

The biometric parameters of the selected GLVs such as *Brassica juncea*, *Trigonella foenum*, *Amaranthus polygonoides*, *Amaranthus tristis* and *Sesbania grandiflora* grown in fresh water on the 45th day were depicted in Table 2.

The first cotyledon emerges within 2 days in *Brassica juncea*, *Trigonella foenum* and *Amaranthus polygonoides* whereas it takes 3 days for *Amaranthus tristis* and *Sesbania grandiflora*. The percentage seed germination was maximum in *Amaranthus polygonoides* and *Trigonella foenum* which was followed by *Brassica juncea*, *Amaranthus tristis* and *Sesbania grandiflora* respectively. At the post harvested stage of the 45th day, the average seedling length (plant height) was found to be maximum for *Sesbania grandiflora* whereas the *Trigonella foenum* had shown the minimum seedling length. Among the GLVs, *Sesbania grandiflora* had recorded the maximum number of leaves which was followed by *Brassica juncea*, whereas the other GLVs recorded less than 100 leaves per plant. A study by Hassan *et al.* (2013) had shown 78 number of leaves in 8 leaf stage of maize similar to *Amaranthus polygonoides* and 200 number of leaves at the milk stage of the maize similar to *Sesbania grandiflora*.

The average surface area of the leaf was measured in graphical squares which were found to be maximum for *Brassica juncea* and minimum for *Trigonella foenum*; and between 6-12 cm² for other GLVs. A similar surface area of the leaf as that of *Brassica juncea* and *Trigonella foenum* was shown in the leaf of *Alnus glutinosa* and *Salix alba* by Juhasz and Loki in 2005.

Among the GLVs, *Amaranthus polygonoides* and *Sesbania grandiflora* had recorded the maximum root length which was followed by *Amaranthus tristis*, *Trigonella foenum* and *Brassica juncea* respectively. The highest shoot length was recorded by *Sesbania grandiflora* and *Brassica juncea* whereas the other GLVs recorded a comparable minimum shoot length. A minimum shoot length was shown in Eurasian watermilfoil by Pinar and Kennedy, (2003) and the maximum shoot length was shown in chrysanthemum plant by Vassilev and Ivanova (2003). Both *Sesbania grandiflora* and *Amaranthus polygonoides* had recorded the maximum root-shoot ratio whereas the minimum root-shoot ratio was recorded by *Amaranthus tristis*.

The maximum root mass was recorded in *Sesbania grandiflora* which was followed by *Brassica juncea* whereas the other GLVs had recorded a comparable root mass. A similar root mass was shown by the study of Vasileva (2012) in *Medicago sativa* L. The highest percentage of dry matter was produced by *Sesbania grandiflora* and the

minimum by *Trigonella foenum*. The other plants of GLVs had shown a comparable dry matter production. The number of seed bunch per plant was maximum in *Brassica juncea* which was followed by *Amaranthus tristis* and *Amaranthus polygonoides* respectively, whereas, the *Trigonella foenum* had shown the minimum seed bunch. A similar study by Gutormson (2002) had shown a seed count of 15.3 in Oats and 20.3 in spring wheat. The distance between the nodes was found to be maximum for *Sesbania grandiflora* which was followed by *Amaranthus tristis* and *Amaranthus polygonoides* respectively, whereas the *Trigonella foenum* and the *Brassica juncea* had shown a comparable distance between the nodes.

The physiological state of the plants of green leafy vegetables had been studied by the assessment of biometric parameters as shown in this study. The maximum growth of vegetable plants was recorded in fresh water (Sangannavar and Kalshetty, 2011).

4.1.2 Qualitative phytochemical analysis of the selected GLVs grown in fresh water

Phytochemicals are virtually present in all the plant tissues of the leaf, the root, the stem and are less concentrated in the fruits and the flowers (Shanthanayaki *et al.*, 2010). Table 3 represents the phytochemical constituents of the methanolic extract of the leaves, the stems and the seeds of the selected green leafy vegetables grown in fresh water.

Carbohydrates in leafy greens occur as sugar monosaccharides (glucose, fructose, arabinose, galactose and rhamnose), disaccharides (sucrose, maltose and trehalose), sugar alcohols (sorbitol, mannitol and xylitol), oligosaccharides (raffinose, stachyose and fructooligosaccharides) and polysaccharides (starch, cellulose, hemi-cellulose and pectins) (Peterbauer *et al.*, 2007).

Proteins are a vital class of biological macromolecule found in all organisms (Giribaldi *et al.*, 2007). Leafy vegetable plant protein constitutes about half the vegetable protein content in the human diet and perhaps contributes more to the total protein (Lefort and Blay, 2011). The color of the natural **quinone** varies from pale yellow to almost black but they create very little contribution to color in higher plants. Their colors are masked by other pigments (Nilesh *et al.*, 2010).

Table 3

Qualitative analysis of the phytochemical constituents of the five selected GLVs grown in fresh water

Name of the Nutrient	<i>Brassica juncea</i>			<i>Trigonella foenum</i>			<i>Amaranthus polygonoides</i>			<i>Amaranthus tristis</i>			<i>Sesbania grandiflora</i>	
	Leaf	Stem	Seed	Leaf	Stem	Seed	Leaf	Stem	Seed	Leaf	Stem	Seed	Leaf	Stem
Carbohydrate	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Proteins	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenol	+	+	+	+	-	+	Tr	Tr	-	-	+	+	+	+
Catechol	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sterols	+	+	Tr	+	+	Tr	+	+	Tr	Tr	Tr	Tr	+	+
Glycosides	-	+	+	-	+	+	-	-	+	+	+	+	-	-
Saponin	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Quinones	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cynogenic glycosides	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	-	+	Tr
Flavonoids	-	+	+	-	-	-	-	-	-	Tr	+	Tr	-	+
Leucoanthocyanidines	-	Tr	-	-	-	-	-	-	-	Tr	-	+	-	Tr
Tannins	+	+	+	+	+	+	-	-	Tr	+	+	-	+	-
Anthocyanins	+	+	+	+	+	+	Tr	Tr	Tr	+	+	+	+	+
Volatile oils	-	+	-	-	-	-	-	-	-	-	Tr	-	-	-
Lignin	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Terpenoids	+	+	+	-	+	+	+	-	+	Tr	-	+	+	+
Cellulose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Free aminoacids	-	+	+	+	+	+	+	+	+	-	+	-	-	+
Starch	-	-	-	-	-	+	-	+	+	-	-	+	-	-
Reducing sugars	+	+	+	-	-	+	-	-	+	-	-	+	-	+

+ Presence - Absence Tr- Trace presence

It was evident from Table 3 that carbohydrate, protein, cellulose and quinone were present in the leaf, the stem and the seeds of all the selected GLVs except the seeds of *S.grandiflora* which was not produced on the 45th day of growth. A recent study by Gopalakrishnan *et al.* (2012) in the ethanolic extracts of *Tylophora pauciflora* had shown the presence of carbohydrate and protein.

Plant **catechols** prevent lipid peroxidation in plasma and erythrocytes (Kidmose *et al.*, 2006). **Saponins** defend plants against microorganisms due to their insecticidal activity and have allelopathic effects on many weeds. Dietary saponins cause a reduction of blood cholesterol, inhibit growth of cancer cells and stimulate the immune system (Francis *et al.*, 2009). There are various anti-nutritional factors (tannins, phytates and oxalates), toxins (**cyanogenic glycosides**) and dietary fibre (cellulose, **lignin**) which, though vital in assessing the overall quality of food, sometimes interfere with the proper assimilation of the other nutrients (Indrayan *et al.*, 2008).

Catechol, saponin, cynogenic glycosides and lignin analysis of the selected GLVs revealed their absence in all the parts of all the extracts of the GLVs, which was in accordance with the findings of Ajayi *et al.* (2011) that the cynogenic glycosides and saponin were absent in *Artocarpus heterophyllus*.

Phenols can help to protect the body against diseases such as heart disease, stroke and certain cancers. Phenols also hold excellent antioxidant properties (Ozen and Kinalioglu, 2008). **Steroids** are adapted triterpenes and have profound importance as hormones, coenzymes and provitamins (Chanda *et al.*, 2008). It was presumed that the regulators of growth and development in plants are different from the hormones that regulate growth and development in animals. It was found that the phytosterols had cholesterol-lowering properties (Latif and Ellal, 2007).

Phenol was present in all the parts of *B.juncea*, except the leaf, the other parts of *A.tristis*, only in the leaf and the seed of the *T.foenum*. Sterol was slightly present in all the seeds of the GLVs and high prominent presence was noticed in the leaf and the stem of *B.juncea*, *T.foenum*, *A.polygonoides* and *S.grandiflora*. An earlier study by Imran *et al.* in 2012 had also shown the presence of sterol in petroleum ether extract and phenol in the methanolic extracts of *Leonotis nepetifolia*.

Glycosides play a significant role in plants and are stored in the form of inactive glycosides. These can be activated by enzyme hydrolysis. Such plant glycosides are used as medications (Brito and Marco, 2007). **Alkaloids** are a group of naturally occurring chemical compounds ubiquitous in the plant kingdom. They have antihypertensive, antipyretic and anti-tumorogenic properties. Alkaloids are also recognized to regulate the plant growth (Jaleel *et al.*, 2007).

Glycosides were absent in the leaf extracts of the GLVs except *A.tristis* and were present in the stem and the seed of *B.juncea* and *T.foenum*. A similar study by Egwaikhide and Gimba (2007) had shown the absence of glycosides in the ethanolic extract of *Plectranthus glandulosus*. Alkaloid was present in all the parts of the *B.juncea*, *T.foenum* and *A.polygonoides* except the seeds of *A.tristis*. The complete absence of flavonoid was noticed in all the parts of the *T.foenum*, *A.polygonoides* and it was present in the stem and the seeds of *B.juncea*, the stem of *S.grandiflora* plants and all parts of *A.tristis*. The absence of alkaloid was also observed in the methanolic extract of *Oxalis corniculata* by Raghavendra *et al.* (2006) and its presence was shown in *Andrographis paniculata* by Shihabudeen *et al.* (2010).

Leucoanthocyanidines are dominant antioxidants and they have potential health effects against cancer, aging, neurological diseases, inflammation, diabetes and bacterial infections (Gowri *et al.*, 2011). **Anthocyanins** are water-soluble vacuolar pigments that may appear red, purple or blue. They take place in all the tissues of higher plants including leaves, stems, roots, flowers and fruits and are powerful antioxidants (Chan *et al.*, 2008).

The leucoanthocyanidines were present only in the seed of *A.tristis* and were present slightly in the stem of *S.grandiflora* and *B.juncea* and the leaf of *A.tristis*. The anthocyanins were present in all the parts of the *B.juncea*, *T.foenum*, *A.tristis* and *S.grandiflora* and were present slightly in all the parts of *A.polygonoides*.

Tannins are found in nearly every part of the plant like the bark, the wood, the leaves, the fruits and the roots. Tannins are involved in many human physiological activities, such as stimulation of phagocytic cells, host mediated tumor activity and wide range of anti-infective actions (Prakash *et al.*, 2009). In plants, **volatiles** are present in

cellular tissues and present in minute amounts which constitute only a small fraction of the total plant weight (Pandey *et al.*, 2006).

Tannin was present in all the parts of *B.juncea* and *T.foenum* and in the leaf and the stem of *A.tristis* and in the leaf of *S.grandiflora*. A study by Amin *et al.* in 2013 had also shown the presence of tannin in the methanolic extract of *Taraxacum officinale*. The volatile oil was absent in all parts of the extracts of the GLVs except in the stem of *B.juncea* and *A.tristis*. A similar study by Raghavendra *et al.* 2006 had also reported the absence of volatile oils in the petroleum ether extracts of *Oxalis corniculata*.

Terpenoids are lipid soluble, large and various class of naturally occurring organic chemicals located in the cytoplasm of the cell. They are used extensively for their aromatic qualities (Swati *et al.*, 2010). **Free aminoacids** play an essential role in physiological functions such as osmoregulation, intracellular pH-buffering capacity, control of enzyme activity (I), neurotransmitter function and inhibition of oxidative reactions (Boldyrev, 2001).

Terpenoids were present in all the GLVs except in the leaves of *T.foenum* and the stems of *A.polygonoides* and *A.tristis*. Earlier study by Ugochukwu *et al.* (2013) in the methanolic extracts of stem bark of *Dennetia tripetala* had also shown the presence of terpenoid. The free aminoacids were present in all the selected GLVs except in the leaf of the *B.juncea*, *S.grandiflora* and the leaf and the seed of *A.tristis*. This was in accordance with the study of Nisha shri *et al.* (2010) that the alcoholic extract of *Corallocarpus epigaeus* had shown the presence of free aminoacids.

Starch is the extensive and abundant storage carbohydrate in plants. Starch is an insoluble glucan composed of two polymers of glucose, amylopectin and amylose. In higher plants, starch is synthesized in plastids by both photosynthetic and non photosynthetic cells (Zeeman *et al.*, 2010).

Starch was absent in all parts of the plants of GLVs except in the seed of *T.foenum* and *A.tristis* and the stem and the seed of *A.polygonoides*. The reducing sugars were present in all parts of *B.juncea* and the seeds of *T.foenum*, *A.polygonoides* and *A.tristis*. Ruchi and Renu,(2010) had also reported the presence of starch and reducing sugar in *Jatropha gossypifolia* L.

Thus the results confirm that the plants of GLVs are rich in phytochemicals. The green leafy vegetables are an immense source of bioactive non-nutritive health promoting factors such as phytochemicals (Sheetal *et al.*, 2008).

4.1.3 Analysis of proximate principles and mineral contents of the selected GLV plants

Moisture content plays a vital role in safe storage and act as a diluent to proteins, minerals and vitamins in the diet (Thiex and Richardson, 2003).

The moisture content of all the selected green leafy vegetables was in-between 12 and 40% which was similar to the results reported in wheat flour (Elena, 2012) and in *Cleome gynendra* (Clement, 2011).

The **ash content** or the ash value, simply represents inorganic salts. These ash values are important quantitative standards (Singh and Sharma, 2010). The ash content of a vegetable provides a measure of the total amount of minerals in it (McClement, 2003). **Crude fiber** is the fiber that remains after food is digested with alkali and acid, which destroys all soluble and some insoluble fiber. It is mainly lignin and cellulose (Islam, 2009).

Among the selected plants of GLVs, *A.polygonoides* was found to be superior with high ash content and the other GLVs recorded comparable values of ash content. Radiotis *et al.* (1996) also recorded similar ash content in *Miscanthus sinensis*. The percentage of crude fiber was found to be maximum in *A.tristis* and minimum in *S.grandiflora*, whereas the other GLVs recorded comparable crude fiber content. According to the findings of Kwenin *et al.* (2011) *Amaranths cruentus* and *Talinum triangulare* had shown a similar ash content as that of *A.tristis* and *B.juncea* respectively.

Available **carbohydrates** are those that are hydrolyzed by enzymes of the human gastrointestinal system to monosaccharides such as sucrose and starch. Monosaccharides require no digestion and can be absorbed directly into the blood stream (Qian *et al.*, 2008). **Proteins** are biochemical compounds consisting of one or more polypeptides typically folded into a globular or fibrous form in a biologically functional way. Proteins provide structural material for the human body and function as enzymes, hormones and antibodies. Dietary proteins are the major source of amino acids (Caliskan and Polat, 2011).

The present study had shown that the total carbohydrate content of *S.grandiflora* was found to be superior, two fold higher than that of *B.juncea* and *T.foenum*, whereas *A.polygonoides* and *A.tristis* recorded a comparable carbohydrate content. The total protein content of *T.foenum* was found to be maximum which was followed by *S.grandiflora*, *B.juncea*, *A.tristis* and *A.polygonoides*. Similar carbohydrate content was reported in *Ceratotheca triloba* and *Wahlenbergia undulata* and similar protein content in *Amaranthus dubius* and *Amaranthus spinosus* by Odhav *et al.* (2007).

The major combustible component of non-food energy in crops is **cellulose** as it has more usable energy than edible energy in crops (Holt and Eric, 2007). The cellulose content of *S.grandiflora* was found to be maximum and minimum in *T.foenum* whereas *A.tristis* and *B.juncea* had shown identical cellulose content. Ververis *et al.* in 2007 had also reported similar cellulose content in Algal biomass.

Iron is an essential trace element found in nearly all living organisms. Iron-containing enzymes and proteins, often containing heme prosthetic group, participate in many biological oxidations and in transport. Iron is the most essential element to the body when chelated to amino acids and is also available for use as a common iron supplement (Hoppe *et al.*, 2010). **Phosphorus** is required for the growth, the maintenance and there pair of all the tissues and cells, and for the production of the genetic building blocks, DNA and RNA. Phosphorus is a component of nucleic acid and as phosphate ester plays an important role in cellular metabolism of other nutrients like carbohydrates, fat etc (Lewis *et al.*, 2006).

In the present study, the highest iron content was noticed in *T.foenum*, the lowest in *S.grandiflora*. The rest of the GLVs recorded a comparable value of iron content. A similar value of iron content was also reported in *Corchorus olitorius* by Orech *et al.* in 2005. The level of phosphorus of all the selected GLVs was found to be similar, which was comparable to the results of Konieczynski and Wesolowski (2007) in *Betula pendula*.

The primary function of **calcium** in plant growth is to provide structural support to the cell walls. **Magnesium** is vital for crop production and is a central atom in chlorophyll molecule, and is involved in photosynthesis. It serves as an activator for many enzymes required in plant growth processes and stabilizes the nucleic acids (Larry, 2011).

All the selected GLVs in this study recorded a comparable level of calcium and magnesium except *S.grandiflora* with the minimum value. A study by Asaolu *et al.*, 2012 had also shown a similar magnesium content as that of *B.juncea* in *Veronia amygdalina*. A similar calcium content as that of *A.tristis* was reported in round variety of *Solanum melanogena* by Agoreyo *et al.* in 2012.

Leafy green vegetables are a significant component of the human diet, providing fibre, minerals and vitamins (Acikgoz, 2011). Minerals are significant in maintaining a good health (Arslan and Ozcan, 2008). This study confirms that the selected GLVs are rich in proximate principles such as the moisture content, ash content, crude fiber, total carbohydrate, total protein and cellulose and mineral contents like iron, phosphorus, calcium and magnesium and it corresponds to the studies by Owolabi *et al.*, (2012) in *Vigna unguiculata*. The results also confirm that the leafy vegetables examined have a high content of crude protein, crude fibre with abundance of minerals and moderate values of ash contents (Asaolu *et al.*, 2012).

4.1.4 Enzymic, non enzymic antioxidants and lipid peroxidation of the selected GLVs grown in fresh water

Antioxidants are compounds that dispose, scavenge and suppress the formation of free radicals or oppose their actions. There are two main categories of antioxidants (enzymic and non enzymic) whose role is to prevent the generation of free radicals that are generated (Surapaneni and Venkataramana, 2007).

Metabolism of oxygen is associated with the formation of reactive oxygen species (ROS) such as superoxide anion radical, hydrogen peroxide which are an inevitable consequence of an aerobic lifestyle. These molecules play an imperative role in the cytotoxic and the mutagenic effects of oxygen and caused oxidation of membrane fatty acids, resulting in lipid peroxidation, oxidation of proteins and DNA damage (Georgiev *et al.*, 2009). Oxidative stress generated by ROS, has been lined to several cellular toxicity processes, including damage to proteins, memberane lipid peroxidation, DNA alteration and enzyme inactivation (Tung *et al.*, 2008). A highly capable antioxidant defense system is present in the plant cells for ROS detoxification including either the non-enzymatic or the enzymatic constituents (Basu *et al.*, 2010).

Superoxide dismutase (SOD) is one of the critical enzymes that protect cells against oxidative damages. It is the key enzyme to diminish the concentration of ROS. SOD catalyses the reduction of superoxide radicals to yield molecular oxygen and hydrogen peroxide. SOD is usually considered to be the first line of defense against oxidative stress (Nan *et al.*, 2006). **Peroxidase** is a significant enzyme and is able to scavenge hydrogen peroxide, which is a major substance degraded by SOD (Liu *et al.*, 2009). Peroxidase is linked with cell wall stiffening and lignification (Patykowski, 2006). Peroxidase catalyses the oxidation of phenolic compounds and certain other closely related substances using oxygen derived from H₂O₂ (Uma and Thripathaiala, 2010).

The present study had shown a maximum activity of SOD in *A.polygonoides* and *S.grandiflora* with a minimum activity in *B.juncea*. A corresponding study by Vijayakumari *et al.*, 2012 had shown similar results in *Cephalandra Indica*. The peroxidase activity was observed to be highest in *A.polygonoides* which was followed by *B.juncea*, *T.foenum*, *A.tristis* and *S.grandiflora* respectively.

Total phenols are the main plant compounds with antioxidant activity. This is believed to be mainly due to their redox properties, which play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Szakiel *et al.*, 2009).

In this study, the total phenol content was found to be maximum in *A.polygonoides* and minimum in *S.grandiflora*, whereas the other GLVs recorded a comparable total phenol content. A similar study in *Ononis natrix* also reported a comparable value of phenol content (Khaled *et al.*, 2007).

Ascorbic acid occurs in all the plant tissues usually being higher in photosynthetic cells and meristems. It is the maximum in the mature leaf, where the chloroplasts are fully developed. Vitamin C has effects on many physiological processes including the regulation of growth, differentiation and metabolism of plants (Shao *et al.*, 2008). **Tocopherol**, a lipid soluble antioxidant is extensively distributed in eight different stereoisomer of which α -tocopherol is known to have the greatest biological activity and act as a potent inhibitor of lipid peroxidation both *in vitro* and *in vivo*. The amount of dietary fat and the food matrix of the meal influence the absorption and bioavailability of vitamin E (Jeanes

et al., 2008). Tocopherols occur widely in plants, but the form of tocopherol often differs in the leaves and the seeds of the same species. α -tocopherol is the primary form of tocopherols in the leaves of plants (Hunter and Cahoon, 2007).

In the present study, *T.foenum* was found to be superior with the maximum value of ascorbic acid followed by other GLVs with the comparable ascorbic acid content. An earlier study also revealed similar ascorbic acid content in cooked cauliflower (Davey *et al.*, 2000). Comparable tocopherol content was noticed in *A.tristis*, *T.foenum* and *A.polygonoides* and was also found to be maximum, whereas the other GLVs, *B.juncea* and *S.grandiflora* had shown comparable value of tocopherol. A study by Azambadrhadad *et al.* (2013) had shown similar tocopherol level in *Elaeagnus angustifoliais*.

H_2O_2 is non-radical reactive oxygen species and the main stable intermediates in the four-electron reduction of O_2 to H_2O (Storch *et al.*, 2010). Shyur *et al.* (2009) reported that the **DPPH** (1,1-diphenyl-2-picryl hydrazyl) scavenging activity had been widely used to evaluate the antioxidant activity of natural products from plants.

H_2O_2 scavenging activity of all the selected GLVs ranges between 65 - 90% with the maximum for *S.grandiflora* and *B.juncea*. Among the selected GLVs, *S.grandiflora* had shown the highest DPPH activity which was followed by *A.polygonoides* and *A.tristis* respectively. The *T.foenum* and *B.juncea* recorded the lowest DPPH activity. Earlier studies had shown similar results in *Hibiscus cannabinus* (Patel *et al.*, 2010) and *Acacia caesia* (Thambiraj and Paulsamy, 2012).

Thus the results clearly indicate the presence of enzymic and non enzymic antioxidants in the selected GLVs which will serve as good sources of antioxidant and free radical scavenging activity. The green leafy vegetables act as antioxidants in the body and the substances in the GLVs has the potential to remove free radicals in the body before they become harmful (Ingrid, 2013).

4.1.5 Pigment levels of the selected GLVs

Photosynthesis is the process, which can harvest solar energy to convert it into chemical energy (starch) in plants. The key molecule for photosynthesis is **chlorophyll**, which absorbs photon, goes to the excited state and transfer the energy to the other pigment molecules, which are in close proximity and in perfect orientation via some membrane

protein complex. These are the prior requirements to funnel the energy (Kubola *et al.*, 2011). **Carotenoids** have attracted immense interest due to their value as antioxidants which have been related to their capacity to reduce cancer and other degenerative diseases (Djaelani *et al.*, 2000).

Among the selected GLVs, *S.grandiflora* was found to be rich in total chlorophyll followed by *B.juncea*. *T.foenum* and *A.polygonoides* were found to be on par with each other. A study by Vassilev and Ivanova, 2003 had shown a similar level of chlorophyll content in chrysanthemum plant. The carotenoid content of all the selected GLVs were found to be comparable to each other. Similar carotenoid content was recorded in Calunga by Simao *et al.*, 2013.

Thus in the present study, the selected GLVs were found to be rich sources of chlorophyll and carotenoid contents. Leafy vegetables also contain several types of photosynthetic pigments that are chlorophylls and carotenoids (Kimura and Rodriguez-Amaya, 2002). Carotenoids and chlorophylls have an important role in the prevention of various diseases associated with oxidative stress, such as cancer, cardiovascular diseases and other chronic diseases (Sangeetha and Baskaran, 2010).

4.1.6 Physico-chemical analysis of the untreated control soil

Soil **pH** refers to its acidity or alkalinity and is the determine of hydrogen ions (H⁺) in the soil. **Texture** is the result of ‘weathering’, the physical and the chemical breakdown of rocks and minerals. Because of the differences in the composition and the structure, materials will endure at different rates, affecting soil’s texture (Ann *et al.*, 2005). Soil electrical conductivity (**EC**) that correlates with soil properties is a measurement that affect crop productivity, including soil texture, cation exchange capacity (CEC), drainage conditions, organic matter level, salinity and subsoil characteristics (Robert *et al.*, 2009).

The soil analysis revealed that the pH of the soil used in this study was within the optimal range and found to be a sandy loamy type of soil. The electrical conductivity was also found to be within the optimal range and lay under salt free condition.

The primary function of **Calcium** after absorption from the soil in plant growth is to provide structural support to the cell walls. Calcium also serves as a secondary messenger when plants are physically or biochemically stressed (Larry, 2011). **Nitrogen** is one of the

important components of proteins, nucleic acids and other cellular constituents of plants and hence it is required for their proper growth and development (Raymond *et al.*, 2004). **Phosphorus** is a major nutrient for crops and serves several significant functions in growth and metabolism like energy, storage and transfer, a component of critical cell constituents, promotion of root development and tillering and facilitating proper maturation (Channakeshava *et al.*, 2006). It is needed by plants to promote root, fruit, flower and seed production. **Potassium** is required by the plants for regulation of water uptake, movement of sugars within the plant, starch production and root growth (Amber, 2011).

The calcium and the nitrogen contents of the present study were below the prescribed optimal range. The phosphorus level in soil was about 10 kg/ha which was in the minimum level of phosphorus as referred by Antonio *et al.* (2013) in the general guide for crop nutrient and limestone recommendation by Iowa university. The potassium level was found to be within the optimal range.

Iron is one of the major abundant minerals in the soil and is unavailable for direct assimilation by plants or microorganisms (Ahamed, 2007). **Copper** is a vital micronutrient for plants, humans and animals as it has a role in many vital biological functions and as a compound in amino acids and proteins. Copper is therefore one of the least movable trace metals. The availability of copper is related to pH, soil type and parent material. **Zinc** is a critical micronutrient to plants and animals. Zinc uptake in plants is a rather common phenomenon and as zinc is phototoxic the effects will most likely be correlated with reduced crop yield and soil fertility degradation (Linus and Erik, 2007).

In the present study, the iron content was found to be 4.4 which was just below the optimal range. The other micronutrients such as manganese, copper and zinc were within the optimal concentration.

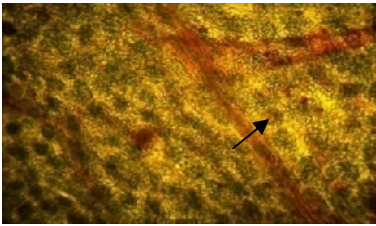
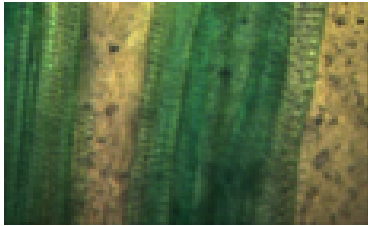
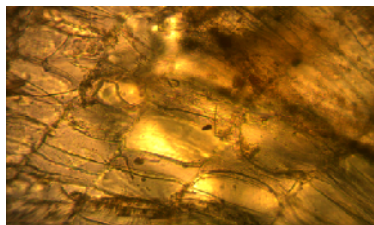
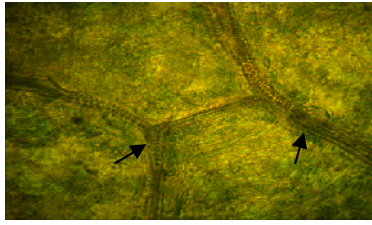
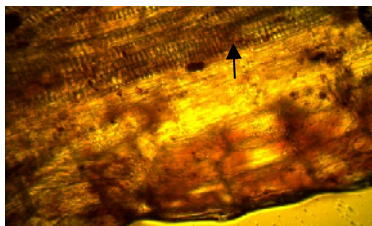
Thus the experimental soil used for the growth of the selected GLVs was rich in macro and micronutrients which in turn would support its growth. A fertile soil with macro and micronutrients with balance of proper nutrients is one that is able to supply the complete dietary needs of the growing plant.

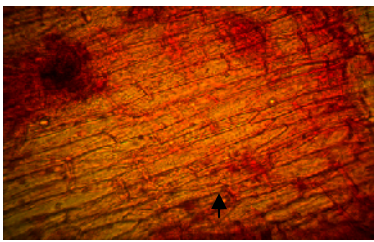
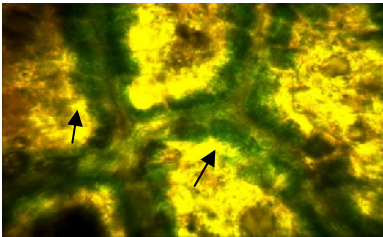
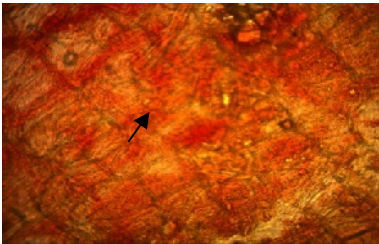
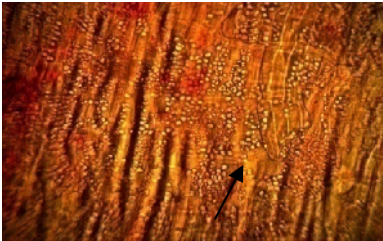
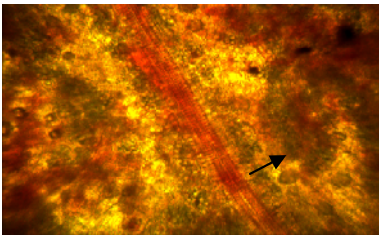
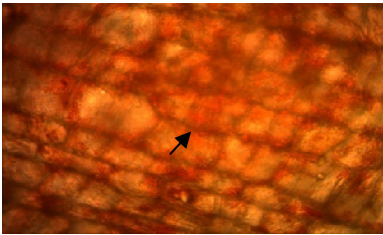
4.1.7 Histological studies of the leaf, the root and the stem of GLVs


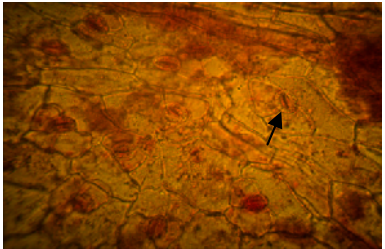
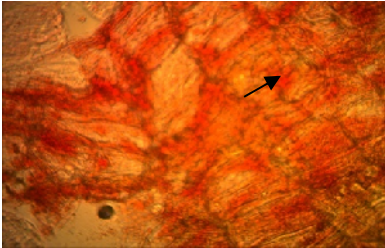
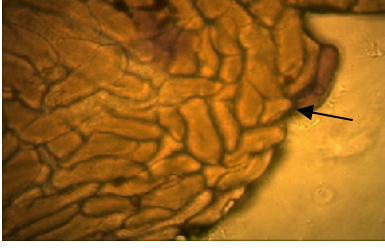
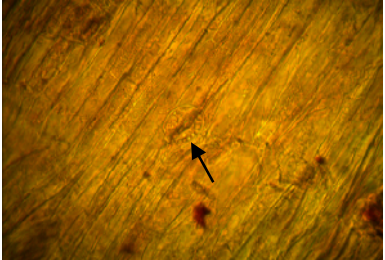
Plate 3 depicts the histology of the leaf, the root and the stem tissues of the selected plants of GLVs grown in fresh water under normal conditions

Plate 3

Histological studies of the selected GLVs

<i>B.juncea</i>	Histological view	Observation
Leaf		Clear cells with the chloroplast were seen in the leaf of <i>B.juncea</i>
Root		The lipid bilayer of the cell membrane was clearly seen
Stem		The intact stem cells were clearly seen
<i>T.foenum</i>	Histological view	Observation
Leaf		The plasmodesmata which acts as a channel was observed
Root		The lipid bilayer of the root was viewed clearly

Stem		The compartment of each cell was clearly observed
<i>A. polygonoides</i>	Histological view	Observation
Leaf		The chlorophyll pigments cells were seen at the edge of the cell
Root		A clear root with some granules was observed
Stem		The stem cells were observed with the granules throughout
<i>A. tristis</i>	Histological view	Observation
Leaf		The veins were seen clearly with chloroplast
Root		A clear view of intact cells was observed

Stem		The cell compartments with granules were seen
<i>S.grandiflora</i>	Histological view	Observation
Leaf		The chloroplasts were observed within the cell
Root		The roots with clear cells were seen
Root nodules		Intact cells were observed in the root nodules
<i>S.grandiflora</i>	Histological view	Observation
Stem		The stem cells with stomata were clearly observed

The sections of the leaf, the root and the stem tissues showed a fine structural integrity which correlates with the findings of Uaboi-Egbenni *et al.* (2009) which stated that

the prepared sections from the plant grown on control revealed properly formed tissues. There was no pathological death observed for all the tissues.

Phase 2

4.2 Characterization of silk dyeing effluent and study of its effect on the growth of selected GLV plants

The silk dyeing effluent was collected from the small scale industrial effluent located at Seelanayakanpatti, Salem. The physico-chemical analysis of the collected crude effluent was carried out. The effluent was diluted to 10% and subjected to degradation using selected biofertilizers such as *Rhizobium sp.*, *Pseudomonas fluorescens* and *Azospirillum sp.* The percentage decolorization of each microbe was analysed in silk dyeing effluent with the co-substrate glucose. Based on the highest percentage decolorization, the two microbes *Pseudomonas fluorescens* and *Azospirillum sp.* were selected to perform the phytohormone quantification. The five selected GLVs were grown in different concentrations of 25%, 50%, 75% and 100% of silk dyeing effluent. As the *T.foenum* plant did not grow in the highest concentrations (100%), the GLVs were grown in 75% effluent and were post harvested on the 45th day and subjected to biometric parameters, phytochemical analysis, proximate principles, mineral contents, antioxidants and pigment analysis. The histological studies of the leaf, the stem and the root of the GLVs grown in the silk dyeing effluent and the physico-chemical analysis of the effluent contaminated soil were also carried out.

4.2.1 Physico-chemical analysis of the untreated silk dyeing effluent

4.2.1.1 Physical examination of the untreated silk dyeing effluent

Generally, the silk dyeing effluent characteristics are needed to give a proper attention to be monitored for avoiding the hazardous environment and to have better safe environmental protections.

Color is thought to be mutagenic, carcinogenic and toxic (Murali *et al.*, 2000). Dye bath effluents, in particular are not only aesthetic pollutants by nature of their color, but may interfere with light penetration in the receiving bodies of water, thereby disturbing biological process. Furthermore, dye effluents include chemicals which are toxic and

carcinogenic (Thanushree *et al.*, 2013). The appearance and the color of the effluent in the study was dark violet at the time of collection and turned turbid later.

Turbidity is an expression of the optical property that causes light to be scattered and absorbed rather than transmitted with no change in direction or flux level through the sample (APHA, 2005).

The turbidity NT units of the present study were 18 for the initial untreated effluent. There was no significant odour and foam in the collected effluent which was in the acceptable limits of no visible discharge of floating solids or persistent foam by Global Effluent requirement (2010).

Total dissolved solids are mainly due to carbonates, bicarbonates, chlorides, sulphates, phosphates, nitrates, nitrogen, calcium, sodium, potassium and iron (Kannan *et al.*, 2009). **Biological oxygen demand** is an index of the oxygen-demanding properties of biodegradable material in water. It is a useful measure for assessing the strength of effluent and its pollution potential. **Chemical Oxygen Demand (COD)** is the measure of the amount of oxygen required by both potassium dichromate and concentrated sulphuric acid to breakdown both organic and inorganic matters (Muhibbu-din *et al.*, 2011). A study by Ogunfowokan *et al.* (2005) reported that the increase in COD could be attributed to an increase in the addition of both organic and inorganic substance from the environment. **Electrical conductivity** is a useful indicator of mineralization and salinity or total salt in a water sample (DWAF, 1996).

In the present study, the total dissolved solid (TDS) concentration was above the acceptable limits of 500mg/l. The EC of 1278 mho/cm was more than the standard limits (300mho/cm). As the total dissolved solid (TDS) value increase, the electrical conductivity value also increases (Siyanbola *et al.*, 2011). The BOD₅ at 20°C and COD analysed were reported to be 2 fold and 3 fold higher than the set limits. An analogous COD value in biogas plant effluent was recorded by Arun kumar (2006). A similar study by Odjegba and Bamgbose, (2012) had shown approximately similar TDS and BOD values in water samples of Ibeshe river polluted with the effluent. Based on the analysis and the comparison of discharge standards of the effluents with the silk industry effluent it has been found that the COD and BOD are not within the limits (Thanushree *et al.*, 2013).

4.2.1.2 Chemical examination of the untreated silk dyeing effluent

The **pH** of the effluent falls within the GER Limits (6-9). Begum *et al.*, (2009) found that alkaline pH could form insoluble hydroxides, oxides and carbonates which also precipitates Cu, Cr, and Zn. **Temperature** is very important, and optimum temperatures tend to induce higher nutrient demands (Resh, 2004). Though the GER limits (2010) is 37°C, the collected effluent from the spot area was 45°C which has come down to room temperature (27°C) after sometime. Increased temperature of water body due to effluent discharge will promote chemical reactions in water (Siyanbola *et al.*, 2011).

The pH alkalinity measured as CaCO₃ was 20mg/l and that of **total alkalinity** was 280mg/l. **pH alkalinity** is a measure of the capacity of the water to neutralise acids and it reflects its so-called buffer capacity (its inherent resistance to pH change). The **total hardness** as CaCO₃ (which is calculated by the sum of calcium and magnesium ion concentration) levels in the untreated effluent was 189 mg/l which was within the WHO standard.

The level of **calcium** (139mg/l) and **magnesium** (50mg/l) was within the desirable limits and the level of sodium was slightly above the standard limits (Water act, 1984). The effluent has about 12mg of potassium per litre. The levels of **iron**, **free ammonia**, **fluoride** and **phosphate** constituents of the effluent were within the standard limits. Venkatesh *et al.* (2009) also reported that the amount of calcium, magnesium, nitrogen and phosphate were low in the different types of industrial effluent.

The most highly oxidized form of nitrogen compounds is commonly present in surface and groundwater because it is the end product of aerobic decomposition of organic nitrogenous matter. The principal forms of nitrogen are organic nitrogen, ammonium (NH₄⁺ or NH₃), nitrite (NO₂⁻) and nitrate (NO₃⁻). The dangers have posed a clear indication that nitrogen must be removed from wastewater before discharge. Nitrogen in untreated wastewaters is primarily in the form of ammonia and organic nitrogen, both soluble and particulate (Kurosu, 2001). The **nitrate** content of the effluent was found to be 26mg/l which was above the prescribed limits. Methemoglobinemia is associated with nitrates above the maximum contaminant level (10 mg/l) as set by the US Environmental protection Agency (EPA, 2006).

The **nitrite** content was found to be low. Nitrate becomes toxic when it is reduced to nitrite, a process that can occur in the stomach as well as in the saliva. **Chloride** is needed for good health and may be important for kidney health, the nervous system and nutrition. There is no known health effects associated with chloride. However, the **sodium** often associated with chloride can be a concern to people suffering from heart disease or kidney disease (Sharmila *et al.*, 2013). The chloride and sulphate levels of the effluent were within the standard limits of EPA (2010). A similar study by Kamlesh *et al.* (2007) reported that a comparable amount of sulphate was found in the distillery, sugar factory mixed effluent. The manganese was not detected in the effluent.

Heavy metals are among the most common environmental pollutants and become an environmental problem concern (Papafilippaki *et al.*, 2007). The Industrial wastewater which could increase heavy metal content in water and sediments on receiving water bodies (Akoto *et al.*, 2008).

Chromium is an essential micronutrient for animals and plants, and is considered as a biological and pollution significant element (Jayana *et al.*, 2009). Chromium in the hexavalent form is very toxic. **Lead** is the most significant of all the heavy metals because it is toxic, very common (Gregoriadou *et al.*, 2001) and harmful even in small amounts.

Nickel is non toxic element but it affects the physiological process at very high concentrations (WHO, 1998). **Zinc** is one of the important trace elements that play a vital role in the physiological and metabolic process of many organisms. Nevertheless, higher concentrations of zinc can be toxic to the organism (Rajkovic *et al.*, 2008).

Cadmium causes adverse changes in the arteries of human kidney. It replaces zinc biochemically and causes high blood pressures, kidney damage etc (Rajappa *et al.*, 2010).

In the present study, the heavy metal lead was not detected in the silk dyeing effluent. The nickel level was above the prescribed limits. The levels of chromium and zinc in the crude silk dyeing effluent were within the set limits and above the prescribed limits respectively. The cadmium of the crude effluent was almost close to the FMENV standard limits, 1998.

Thus, the study has revealed that the unsafe crude silk dyeing effluent from small scale industries is found to have organic, inorganic compounds and heavy metals. Some

heavy metals such as Cr, Pb, Ni, Zn and Cd were present in the effluent from the industries and had been found to be carcinogenic and poisonous depending on the dose (Tamburlini *et al.*, 2002). The dye effluents due to the lack of financial technologies to clean up the effluents when discharged into the environment streams leads to serious environmental and health concerns (Fang *et al.*, 2004).

4.2.1.3. Bioassay test

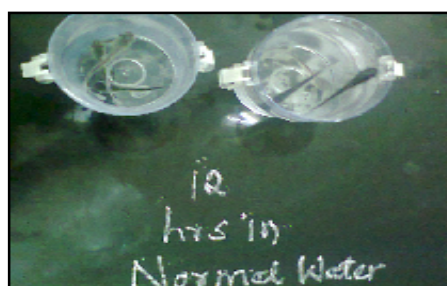
Plate 4 represents the bioassay test in fresh water and crude silk dyeing effluent (100%).

Plate 4

Bioassay test in fresh water and crude silk dyeing effluent



N 1



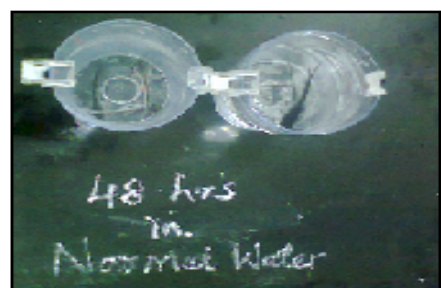
N 2



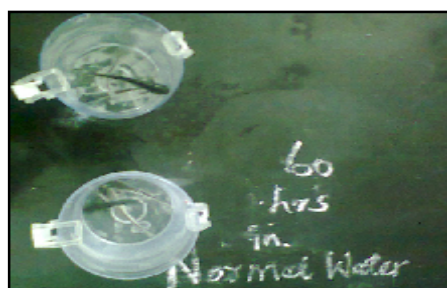
N 3



N 4



N 5



N 6



N 7



N 8

Plate 4 (Contd...)



N 9



E1



E 2

- N 1 - 9 - Fish grown in fresh water observed from 0 hr-96 hrs. The pictorial representation confirms 0% mortality.
- E 1 - 2 - Fish grown in the crude silk dyeing effluent water within 0 hr-12 hrs. The pictorial representation confirms the 100% mortality.

Heavy metals and chemicals are metabolic inhibitors of living organisms. They exert toxic effects in the organisms at tissue, cellular, sub cellular and molecular levels which interact with the protein leading to denaturation, precipitation and enzyme inhibition.

It was clear from Plate 4 and Table 10 that the fish allowed to grow in fresh water survived till 96 hrs of experimental period whereas those in crude effluent survived only upto 12 hrs. Similar results were found in *Labeo rohita* fish exposed to 0.5mg/ml of copper and zinc with 100% mortality (Vineeta *et al.*, 2006). The effluent is capable of causing death of some fish and macro-invertebrates because the oxygen required by these aquatic habitats will be consumed by micro organisms (APHA, 1995) and decrease the oxygen consumption of the fish (Fabian *et al.*, 2000).

4.2.2 Decolorization of the silk dyeing effluent

Many microorganisms belonging to different taxonomic groups such as bacteria had been reported for their ability to decolorize the dyes (Chen *et al.*, 2003). Plate 5 depicts the cultures of the three different microorganisms namely *Rhizobium sp.*, *Pseudomonas fluorescens* and *Azospirillum sp.* used in this study.

Nutrient constituent in the medium have marked effect on color removal along with natural supplements had a positive impact on dye decolorization (Palanivelan *et al.*, 2013).

4.2.2.1 Preliminary decolorization of the silk dyeing effluent

Figure 2 and Plate 6 represents the preliminary studies on percentage decolorization of silk dyeing effluent (with minimum concentrations) by *Pseudomonas fluorescens*, *Azospirillum sp.* and *Rhizobium sp.*, respectively for a period of 20 days with an interval of 5 days.

The percentage decolorization was found to be least in *Rhizobium sp.*, with 52% decolorization on the 20th day. The maximum decolorization was observed in *Pseudomonas fluorescens* followed by *Azospirillum sp.* A study by Elias *et al.* (2000) had also shown similar decolorization % in reactive blue dye (84.4%) by *Trametes hirsute*. Among the three biofertilizers used, the *Pseudomonas fluorescens* was found to be more effective in degradating silk dyeing effluent. Figure 2 depicts the comparison of preliminary decolorization of silk dyeing effluent with different microorganisms.

The cultivated *Pseudomonas fluorescens* from King's B medium and *Azospirillum sp.*, from Azospirillum medium were inoculated in the nutrient slants. Plate 5 represents the same.

Figure 2

Preliminary decolorization of the silk dyeing effluent by *P. fluorescens*, *Azospirillum sp.* and *Rhizobium sp.*

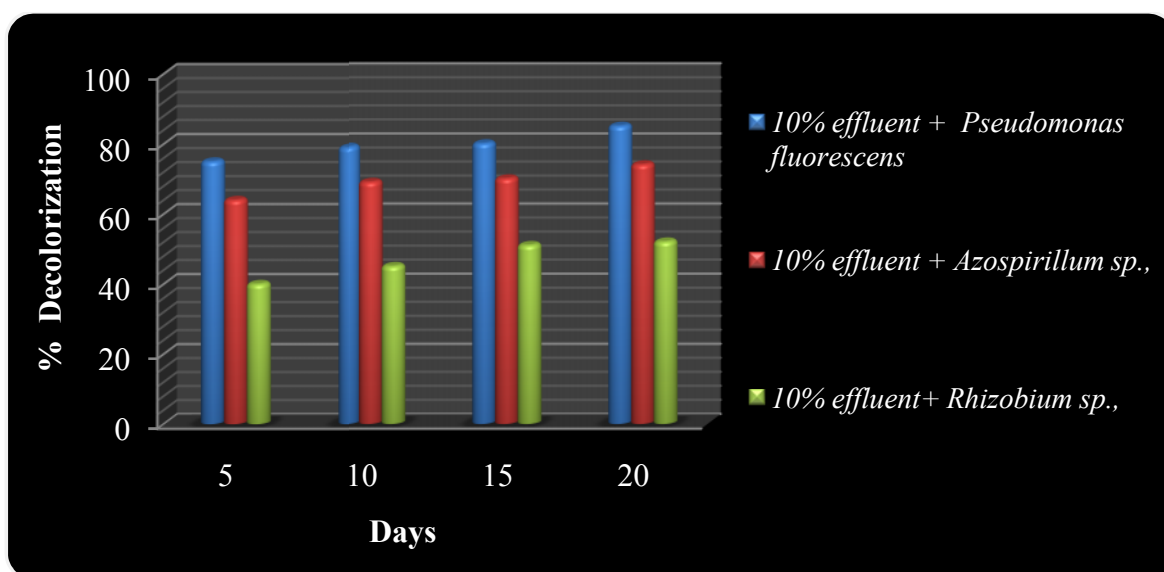


Plate 5

Cultures preserved to perform degradation



PF 1

PF 2

A 1

PF 1 - *Pseudomonas fluorescens* cultures in UV transilluminator

PF 2 - *Pseudomonas fluorescens* maintained in nutrient slants

A 1 - *Azospirillum sp.*, maintained in nutrient slants

Plate 6

Preliminary studies on decolorization of the silk dyeing effluent in different concentrations by *P.fluorescens*, *Azospirillum sp.* and *Rhizobium sp.*



Pseudomonas fluorescens, *Azospirillum sp.* and *Rhizobium sp.*,
inoculated in 10% effluent

4.2.2.2 Percentage decolorization of effluent by *Rhizobium sp.*, *Azospirillum sp.* and *Pseudomonas fluorescens*

The decolorization of silk dyeing effluent of different concentrations (25%, 50%, 75% and 100%) by *Rhizobium sp.*, *Azospirillum sp.* and *Pseudomonas fluorescens* with the co-substrate glucose was depicted in Figure 3 and Plate 7.

The percentage decolorization was improved by the addition of glucose (0.002g) as co-substrates but with the increasing concentrations of the effluent, the percentage decolorization decreases. About 75% decolorization was read in 25% effluent which in turn reduced by 25% and reaches to 50% of decolorization in 100% effluent. The percentage of decolorization was reduced with increasing concentrations of the effluent. Thus the highest percentage of decolorization was evaluated in 25% effluent and the lowest in 100% effluent.

Similar percentage decolorization was found in acid blue by *Trametes hirsute* (Elias *et al.*, 2000). A similar study by Tripathi and Srivastava (2011) had shown 90 % decolorization of acid orange 10 by *Pseudomonas putida*. Wang *et al.* (2009) reported 96.2% decolorization of reactive red 180 anaerobically by *Citrobacter sp.*, when added with glucose at 4 g l⁻¹.

Figure 3

Percentage decolorization of the silk dyeing effluent by *P. fluorescens*, *Azospirillum sp.* and *Rhizobium sp.*, with the co-substrate (glucose)

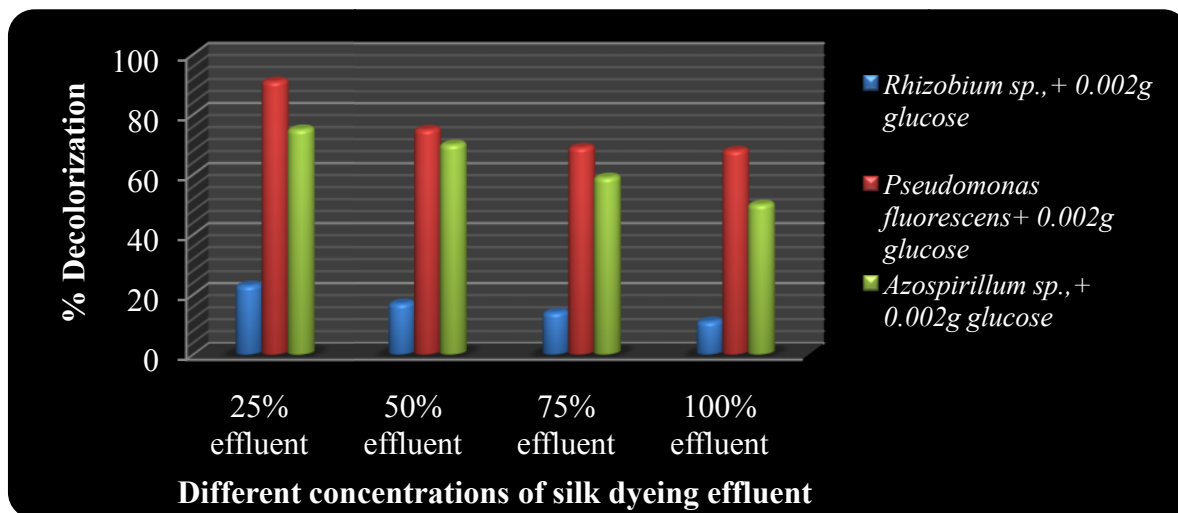


Plate 7

Decolorization of the silk dyeing effluent with different concentrations by *P. fluorescens*, *Azospirillum sp.* and *Rhizobium sp.*, with the co-substrate (glucose)



Rhizobium sp., with different concentrations of the effluent (25% -100%)



P. fluorescens with different concentrations of the effluent (25%-100%)



Azospirillum sp., with different concentrations of the effluent (25%-100%)

The microbial decolourization could be a viable means in ridding dye waste water. Dye molecule absorption into the cell surface appears to be quick and is often completed in some hours. The direct reactive dyes could all be cleared out of solution using the same approach (Palamthodi *et al.*, 2011). In the present study, all the three microorganisms were effectively decolorized the effluent at the lower concentration and has been reduced with the increasing concentration of the effluent. Among the microbes, *Rhizobium sp.*, was found to be less effective in decolorizing the effluent and hence the other two microorganisms (*Pseudomonas fluorescens* and *Azospirillum sp.*) were selected for further study.

4.2.3 Production of phytohormones by selected biofertilizers (*Pseudomonas fluorescens* and *Azospirillum sp.*)

Table 4 and Plate 8 illustrate the level of phytohormones (Indole acetic acid and Gibberellic acids) in silk dyeing industrial effluent inoculated by *Pseudomonas fluorescens* and *Azospirillum sp.*

Table 4

Production of phytohormones by selected biofertilizers

Inoculum in Silk dyeing industrial effluent	IAA($\mu\text{g } 25\text{ml}^{-1}$)	GA ₃ ($\mu\text{g } 25\text{ml}^{-1}$)
<i>Pseudomonas fluorescens</i>	78.0	6.9
<i>Azospirillum sp.</i> ,	70.4	7.1

Plate 8

Production of IAA and GA₃ by *P. fluorescens* and *Azospirillum sp.*, in silk dyeing effluent



The IAA production by *Pseudomonas fluorescens* and *Azospirillum sp.*, in the silk dyeing industrial effluent was found to be $78\mu\text{g } 25\text{ml}^{-1}$ and $70.4\mu\text{g } 25\text{ml}^{-1}$ respectively. The biofertilizers *Pseudomonas fluorescens* and *Azospirillum sp.* synthesizes about $6.9\mu\text{g } 25\text{ml}^{-1}$ and $7.1\mu\text{g } 25\text{ml}^{-1}$ of GA₃.

Thus the study confirms that the selected biofertilizers (*Pseudomonas fluorescens* and *Azospirillum sp.*) produced more IAA than GA₃. The beneficial bacteria referred as plant growth promoting bacteria (PGPB), which support the growth via production of phytohormones (Bai *et al.*, 2002). The most efficient PGPB studied for their ability to produce phytohormones are *P. putida*, *P. fluorescens*, *Azospirillum spp.* and *Bacillus spp.* (Ahamed, 2007).

4.2.4 Biometric analysis of the selected GLVs grown in silk dyeing effluent

The growth of the GLV, *B.juncea* grown in different concentrations (25%, 50%, 75% and 100%) of the effluent on the 45th day was depicted in Plate 9.

4.2.4.1 Biometric parameters of *Brassica juncea* grown in silk dyeing effluent

The first cotyledon emerges within 8 days in *B.juncea* grown in different concentrations of the effluent. It may be due to the interaction of the effluent with the seeds which has delayed the germination. The average percentage of seed germination was analyzed to investigate the difference between the effect of effluent on plants grown in

different concentrations of effluent (25%, 50%, 75% and 100%). The results clearly indicate that the percentage of seed germination gradually decreased with the increasing concentrations of the effluent. Thus the concentrations of the effluent and the percentage germination were inversely proportional. The seedling length (cms) was about 7 fold higher in *B.juncea* plant grown in 25% concentration of the effluent and 6 fold higher in 50% concentration of the effluent than grown in 100% concentration of the effluent. Thus the seedling growth was drastically reduced with the increasing concentration of the effluent.

A study by Medhi *et al.* in 2008 also reported that there was a significant decrease in seed germination, root length and shoot length with increasing concentrations of the effluent.

The average number of leaves in the *B.juncea* plant grown in effluent was higher at the lower concentrations of the effluent and very less at the higher concentrations of the effluent.

Plate 9

***Brassica juncea* grown in different concentrations of the silk dyeing effluent**



BJE (BJA, BJB)

***Brassica juncea* grown in 25% effluent (BJA)**

***Brassica juncea* grown in 50% effluent (BJB)**



Brassica juncea grown in 75% effluent (BJC)



Brassica juncea grown in 100% effluent (BJD)

Uaboi-Egbenni *et al.* in 2009 also reported that the effluents affected the stem length in *Abelmoschus esculentus*. The average root length and shoot length were corresponding to the seedling length of the plants grown in different concentrations of the effluent. The root shoot ratio of plants grown in various concentrations of the effluent was below 1.0. The average root mass of the *B.juncea* plants grown in different concentrations of the effluent ranges between 3-4g. The dry matter content of the plant decreases with the increasing concentrations of the effluent. The seed bunch was completely affected by various concentrations of the effluent. The increasing concentrations of the effluent did not affect the distance between nodes (cm).

4.2.4.2 Biometric parameters of *Trigonella foenum* grown in the silk dyeing effluent

The growth and biometric parameters of *T.foenum* grown in different concentrations (25%, 50%, 75% and 100%) of the silk dyeing effluent was represented in Plate 10.

Plate 10

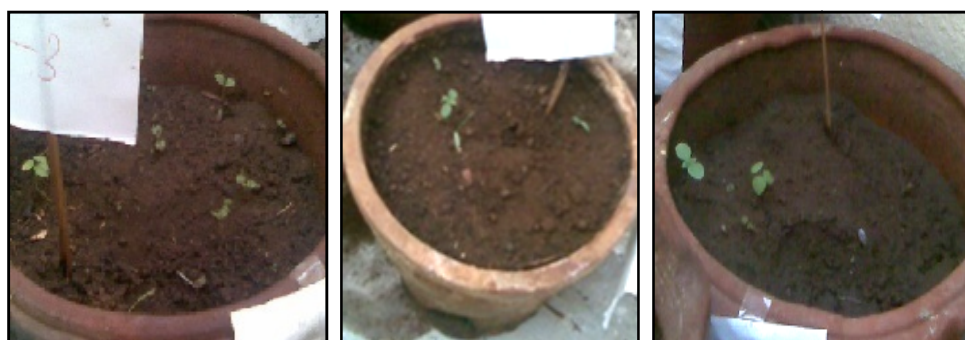
Trigonella foenum grown in different concentrations of the silk dyeing effluent



Trigonella foenum in 25% effluent (TFA)

Trigonella foenum in 75% effluent (TFC)

Trigonella foenum in 50% effluent (TFB)



Trigonella foenum in 100% effluent (TFD)

The *T.foenum* plants grown in 25% and 50% concentrations of the silk dyeing effluent took about 2 days for the first cotyledons to germinate whereas the 75% and 100% crude effluents delayed the germination by one more day. The percentage of seed germination was very high for the *T.foenum* plants grown in fresh water whereas it was significantly reduced when grown in various concentrations of the silk dyeing effluent. The various concentrations of the silk dyeing effluent also reduced the seedling length and number of leaves of the *T.foenum* plant. Even though the seed germinated in 100% concentration of effluent, the *T.foenum* plants did not grow further. Thus 100% concentration of the effluent did not support the growth of *T.foenum* plants.

The average number of leaves had a significant difference from TFN plant grown in fresh water whereas the different treatment of silk dyeing effluent has reduced the number of leaves in TFA, TFB and TFC (45 ± 4.08 , 42 ± 2.1 , 36 ± 0.014). The plant went dry without

leaves in 100% effluent. It is therefore reasonable to presume that 100% crude effluent did not support the growth of the *T.foenum* plants.

The surface area of the leaves, the dry matter and the distance between the nodes did not vary much between the plants grown in different concentrations of the effluent. A comparable value of root length, shoot length, root-shoot ratio, root mass, number of seed bunch per plant were noticed in *T.foenum* plant grown in 25% and 50% concentrations of the effluent whereas they were reduced drastically in 75% concentrations of the effluent. A similar study by Rajendra babu *et al.*, (2006) shows a decrease in percentage of the seed germination, the seedling growth, the root length and the shoot length with increasing effluent concentrations in black gram (*Vigna mungo*). Thus the increasing concentrations of the effluent did not support the growth of the *T.foenum* plants as revealed in Plate 10.

4.2.4.3 Biometric parameters of *Amaranthus polygonoides* grown in silk dyeing effluent

Plate 11 gives the biometric parameters of *Amaranthus polygonoides* grown in different concentrations (25%, 50%, 75% and 100%) of the silk dyeing effluent.

Plate 11

Amaranthus polygonoides grown in different concentrations of the silk dyeing effluent



Amaranthus polygonoides in 25% effluent (APA)



Amaranthus polygonoides in 50% effluent (APB)



Amaranthus polygonoides in 75% effluent (APC)



Amaranthus polygonoides in 100% effluent (APD)

The *A. polygonoides* plants treated with different concentrations of the silk dyeing effluents took 3 days to germinate. The percentage seed germination, the seedling length, number of the leaves per plant, the root length, shoot length and the root- shoot ratio were reduced in plants with the increasing concentrations of the effluent, whereas, the surface area of the leaves, the root mass, the dry matter and the distance between the nodes did not vary much among the plants grown in various concentrations of the effluent. The seed bunch appears only in plants grown in lower concentrations (25% and 100%) of the effluent.

As indicated in Plate 11 higher concentrations of the effluent affected the growth of the *A. polygonoides* plant.

4.2.4.4 Biometric parameters of *Amaranthus tristis* grown in the silk dyeing effluent

The appearance of first cotyledon grown in different concentrations of silk dyeing effluent delayed 2 days when compared to the same plant grown in fresh water. The percentage seed germination, the seedling length, number of the leaves per plant, the root length, the root-shoot ratio and the dry matter of the *A. tristis* plants were significantly reduced with the increasing concentrations of the effluent. The shoot length and root mass were similar for the plant grown in 25%, 50%, 75% and 100% concentrations of the effluent whereas it was significantly reduced in plants grown in 100% concentration. The effluent did not affect the seed bunch of *A. tristis* plant as it was numerous for all the concentrations of the effluent. Thus increasing the concentrations of the effluent affected the growth of *A. tristis* plants as evidenced in Plate 12.

Plate 12

Amaranthus tristis grown in different concentrations of the silk dyeing effluent



Amaranthus tristis in 25% effluent (ATA)
Amaranthus tristis in 50% effluent (ATB)

Amaranthus tristis in 75% effluent (ATC)



Amaranthus tristis in 100% effluent (ATD)

4.2.4.5 Biometric analysis of *Sesbania grandiflora* grown in the silk dyeing effluent

Plate 13 represent the biometric parameters and growth of the *Sesbania grandiflora* grown in different concentrations (25%, 50%, 75% and 100%) of the silk dyeing effluent.

Without any disparity all the plants had their first germination within 3 days similar to plants grown in fresh water. It was clear from the table 16 that the percentage seed germination, the seedling length, number of leaves per plant, the root length, the shoot length and the dry matter content of *S.grandiflora* plants were gradually reduced with increasing concentrations of the silk dyeing effluent. The parameters such as surface area of the leaves, the root shoot ratio, the root mass and the distance between the nodes of *S.grandiflora* plants did not vary much between the different concentrations of the effluent.

Thus in the growth studies of selected GLVs, the biometric parameters were affected with increasing concentrations of the silk dyeing effluent. Growth analysis is functions of a large number of metabolic processes, which are affected by the environmental factors (Caglar *et al.*, 2010). The effluent water and polluted soil decreases the growth of vegetables, this may be due to their toxicity (Sangannavar and Kalshetty, 2011).

Plate 13

Sesbania grandiflora grown in different concentrations of the silk dyeing effluent



Sesbania grandiflora in 25% effluent (SGA)



Sesbania grandiflora in 50% effluent (SGB)



Sesbania grandiflora in 75% effluent (SGC)



Sesbania grandiflora in 100% effluent (SGD)

4.2.5 Qualitative phytochemical analysis of the methanolic extract of the selected GLVs grown in 75% of silk dyeing effluent

Among the selected GLVs grown in crude silk dyeing effluent, *T.foenum* did not survive on the 45th day of post harvested stage. So, methanolic extracts of the leaf, stem and seeds of GLVs grown in 75% of silk dyeing effluent were subjected to phytochemical studies. The *S.grandiflora* seeds were not analyzed as they were not produced within the experimental period (45 days).

It clearly indicates the presence of carbohydrate, proteins, sterols, quinones, anthocyanin and cellulose in the leaves, the stem and the seeds of selected GLVs. The catechol, saponin, cyanogenic glycosides and lignin were absent in all parts of the selected GLVs.

The phenol content was present in the leaves, stem and seeds of all the GLVs except the stem of *T.foenum*, the seed of *A.polygonoides* and the leaf of *A.tristis*. Glycosides were absent in the leaf of all the GLVs except *A.tristis* and present in the stem and the seeds of *B.juncea*, *T.foenum* and *A.tristis*. Flavonoid is present only in the stem and the seed of *B.juncea*, the stem of *S.grandiflora* and all parts of *A.tristis*. Flavonoids were completely absent in *T.foenum* and *A.polygonoides*. Leucoanthocyanidines were slightly present in the stem of *B.juncea* and *S.grandiflora* and in the leaf of *A.tristis*, the other GLVs did not contain the same. Tannins were present in the leaves, the stem and the seeds of *B.juncea* and *T.foenum* and in the leaf and stem of *A.tristis*, the leaf of *S.grandiflora* and trace in the seed of *A.polygonoides*. Only the stem of *B.juncea* and *A.tristis* shows the presence of volatile oil. Terpenoids were present in all the selected GLVs except the leaf of *T.foenum* and the stem of *A.polygonoides* and *A.tristis*. Except the leaf of *B.juncea* and *S.grandiflora* and the leaf and seed of *A.tristis*, all the other parts of GLVs shows the presence of free aminoacids. The starch was present only in the seeds of *T.foenum*, the stem and the seeds of *A.polygonoides* and the seeds of *A.tristis*. The reducing sugar was present in all parts of *B.juncea*, in the seed of *T.foenum*, *A.polygonoides* and *A.tristis*.

Thus the phytochemical constituents of the selected GLVs were affected by the silk dyeing effluent treatments which also correlate with the findings of Watermann and Mole (1994) that the content and composition of phytochemicals may change as a consequence of chemical treatment (effluent) and climatic stress.

Table 5

Qualitative analysis of phytochemical parameters of the selected GLVs grown in 75% of the silk dyeing effluent

Name of the Nutrient	<i>B.juncea</i>			<i>T.foenum</i>			<i>A.polygonoides</i>			<i>A.tristis</i>			<i>S.grandiflora</i>	
	Leaf	Stem	Seed	Leaf	Stem	Seed	Leaf	Stem	Seed	Leaf	Stem	Seed	Leaf	Stem
Carbohydrate	+	Tr	Tr	+	Tr	Tr	+	+	+	+	Tr	+	+	+
Proteins	+	Tr	Tr	+	Tr	Tr	+	Tr	+	+	Tr	+	+	+
Phenol	+	+	+	+	-	+	Tr	Tr	-	-	+	+	+	+
Catechol	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sterols	+	+	Tr	+	+	Tr	+	+	Tr	Tr	Tr	Tr	+	+
Glycosides	-	+	+	-	+	+	-	-	+	+	+	+	-	-
Saponin	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Quinones	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cynogenic glycosides	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkaloids	+	Tr	+	+	Tr	+	+	Tr	+	+	+	-	+	Tr
Flavonoids	-	Tr	+	-	-	-	-	-	-	Tr	Tr	Tr	-	Tr
Leucoanthocyanidines	-	Tr	-	-	-	-	-	-	-	Tr	-	+	-	Tr
Tannins	+	+	+	+	+	+	-	-	Tr	+	+	-	+	-
Anthocyanins	+	Tr	+	+	Tr	+	Tr	Tr	Tr	+	Tr	+	+	+
Volatile oils	-	Tr	-	-	-	-	-	-	-	-	Tr	-	-	-
Lignin	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Terpenoids	+	+	+	-	+	+	+	-	+	Tr	-	+	+	+
Cellulose	+	Tr	+	+	+	+	+	Tr	+	+	Tr	+	+	+
Free aminoacids	-	+	+	+	+	+	+	+	+	-	+	-	-	+
Starch	-	-	-	-	-	+	-	+	+	-	-	+	-	-
Reducing sugars	+	+	+	-	-	+	-	-	+	-	-	+	-	+

+ Presence - Absence Tr - Trace presence

4.2.6 Proximate principles and mineral contents of the selected GLVs grown in 75% of silk dyeing effluent

As *T.foenum* plants did not survive in 100% silk dyeing effluent, the selected green leafy vegetables grown in 75 % effluent were subjected to proximate principles and mineral contents on the 45th day of growth.

Among the selected GLVs, the *A.tristis* had recorded the maximum moisture content which was followed by *S.grandiflora*, *A.polygonoides*, *B.juncea* and *T.foenum* respectively. A maximum level of ash content was noticed in *A.polygonoides* when compared to other GLVs. Similar to the moisture content, the percentage crude content was also found to be maximum in *A.tristis*, the other GLVs were recorded a comparable crude fiber except the *B.juncea* with a minimum value. The total carbohydrate observed in *B.juncea*, *T.foenum* and *A.polygonoides* were very low when compared to *S.grandiflora* and *A.tristis*, which were shown an approximately 2 fold higher carbohydrate content. A study by Odhav *et al.* (2007) had also shown comparable values of carbohydrate content in *B.juncea* and *T.foenum*. A comparable total protein and cellulose content were recorded in all the GLVs which were found to be 2 fold lower than the protein content of GLVs grown in fresh water.

A maximum level of iron content was noticed in *T.foenum* when compared to the other GLVs which were recorded comparable iron content. The phosphorus content was found to be 1-2mg/g with a maximum level in *A.tristis* and *B.juncea*. This was in accordance with the results in *Hypericum perforatum* by Konieczynski and Wesolowski (2007). The maximum level of calcium and magnesium were detected in *B.juncea*. The minimum level of calcium and magnesium was recorded in *A.polygonoides*.

Thus the proximate principles and mineral contents of all the GLVs were reduced to certain extent when compared to the GLVs grown in fresh water. According to the study by Khan and Richa, 2012, the maximum reduction in carbohydrate and protein were found in Tomato plant grown in industrial effluents. Earlier studies had shown a decreased protein content in seeds of maize exposed to effluents (Singh *et al.*, 2002). A study by Rahman

and Inden (2012) also revealed that the mineral contents in *Capsicum annum* were significantly affected by effluent treatments.

4.2.7 Enzymic, non enzymic antioxidants and lipid peroxidation of the selected GLVs grown in 75% of silk dyeing effluent

The activity of SOD was found to be maximum in *A.polygonoides*, *S.grandiflora* and *A.tristis* and minimum in *T.foenum* and *B.juncea*. Similar results were noticed in *Terminalia chebula* by Vijayakumari *et al.*, (2012). A significant difference in the peroxidase activity of the GLVs was noticed with the maximum activity in *A.polygonoides* and *S.grandiflora* and minimum activity in other GLVs. An approximately similar peroxidase activity was observed in *Helianthus annulus*. L by Groppa *et al.* in 1999.

The total phenolic content was found to be maximum in *A.tristis* and *A.polygonoides* and minimum in *T.foenum* whereas *B.juncea* and *S.grandiflora* had shown a comparable phenolic content. Khaled *et al.* (2007) also noticed a minimum phenolic content in *Cicer aretinum* and maximum in *Linum pubescans*. The *A.tristis* and *A.polygonoides* had shown a comparable ascorbic acid content and the same was found to be identical *B.juncea* and *S.grandiflora*. A minimum ascorbic acid content was recorded in *T.foenum*. The tocopherol content was observed in the selected GLVs did not vary much.

The free radical scavenging assay of H₂O₂ of the selected GLVs was in the range of 50-79%. Among the GLVs, *S.grandiflora* had shown the highest H₂O₂ scavenging activity. A comparable DPPH activity was noticed in all the GLVs with the maximum activity for *B.juncea*. A similar study by Thambiraj and Paulsamy (2012) in *Acacia caesia* and Lim and Rabeta, (2013) in water apple also noticed a comparable DPPH activity.

Thus the enzymic and non-enzymic antioxidants and lipid peroxidation of the selected green leafy vegetables were affected by the effluent compared to their growth in fresh water. According to the study by Fatima and Ahmad (2005), the effluent treatment of the onion bulbs (*Allium cepa* L.) resulted in significant decrease in the activities of enzymic antioxidants at higher concentrations of the effluent. Antioxidants were studied as biomarkers of the stress induced by treatment with effluent (Sangeetha *et al.*, 2012).

4.2.8 Pigment levels of the selected GLVs grown in 75% of silk dyeing effluent

The total chlorophyll level of *S.grandiflora* (2.02 mg/g) was found to be higher when compared to the other GLVs which were recorded a comparable chlorophyll values. The minimum level of chlorophyll was found in *T.foenum*.

The amount of carotenoids was found to be higher in *A.tristis* and *A.polygonoides*, the other GLVs were recorded a minimum level. Similar investigation by Sangannavar and Kalshetty, (2011) proved that the GLVs such as palak, mentya, sabasagi, kotambari, mulangi, pundi grown in effluent showed lower chlorophyll content compared to the control. A study by Saskia *et al.* in 2013 also had shown similar carotenoids level in Orange.

Thus the GLVs grown in effluent proved to have lower chlorophyll and carotenoid contents which supports that the total chlorophyll and carotenoids were reduced in tomato was affected by the higher concentrations of the effluents (Kaushik *et al.*, 2004).

4.2.9 Analysis of the soil contaminated with silk dyeing effluent

The **pH** (8.1) and **EC** (1.2) noticed in effluent soil was above the standard limits. Similar results were also reported in the soil sample collected in the sanganer region in the month of august by Nidhi and Ashwani in 2011.

The soil **texture** was observed to be S1- type sandy clay and loam. All the macronutrients (N, P, K and Ca) and micronutrients (Fe, Cu and Zn) except manganese in the effluent soil were detected to be lower than the optimal range. The results indicate that the effluent reduces the level of both the macronutrients and micronutrients below the optimal range. **Calcium** can be lost from the soil in contaminated water and its deficiency symptoms contain death at the growing point, abnormally dark green foliage, weakened stems, shedding flowers (Larry, 2011). **Nitrogen** deficiency in the soil affects the cellular metabolism and may lead to dwarfism of the plant, decrease of chlorophyll content of the cells and finally may lead to plant death (Raymond *et al.*, 2004). The alkaline pH of the effluent medium bath can also be the cause of low level of **copper**, as heavy metals are precipitated as their salts at high pH and are deposited as sediments (Rao and Manjula, 2000).

Thus from this study, the effluent treated soil will not be able to provide the macronutrients and micronutrients for the growth of the GLVs. According to the study by Rena and Kanika (2013), the application of industrial effluent affects the physico-chemical properties of the soil as well as the fertility of soil.

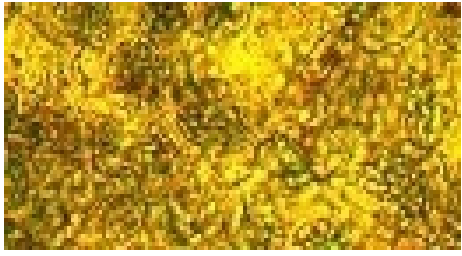
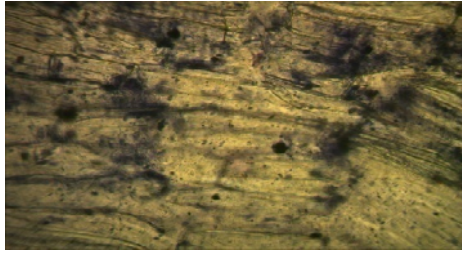
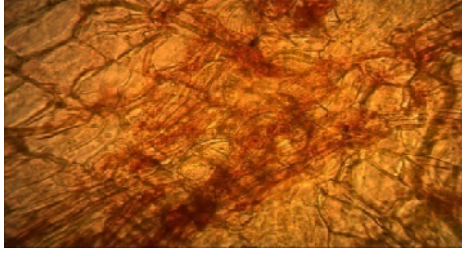
4.2.10 Histological studies of the leaf, the root and the stem sections of the GLVs grown in highest concentration of the effluent at which the GLVs survived

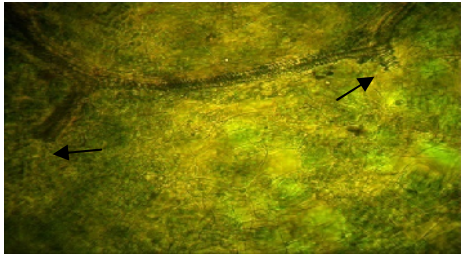
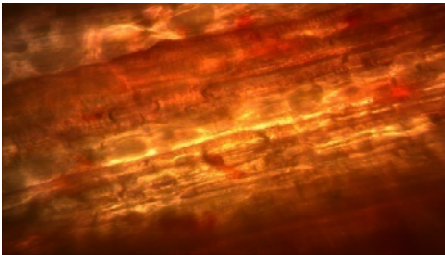
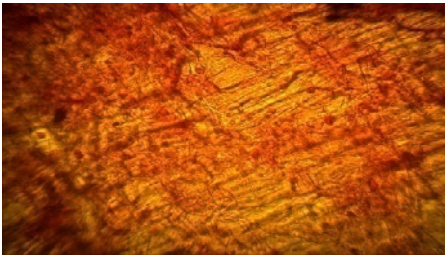
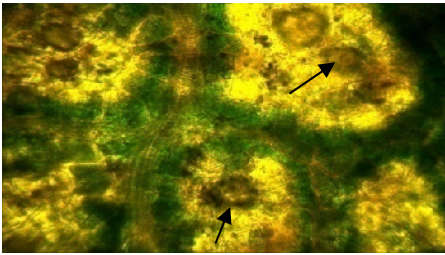
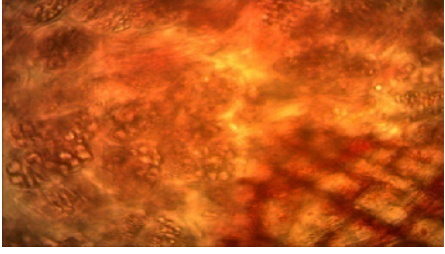
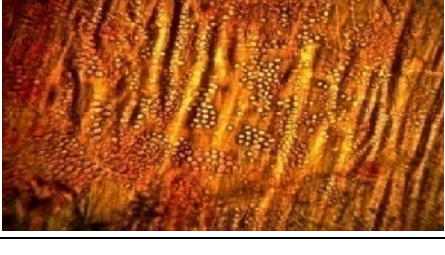
Cytological studies of the leaf, the root tip cells and the stem grown in the industrial effluent was known to induce cellular abnormalities in plants (Tharakeshwari and Shobha, 2006). The leaf, the root and the stem of the selected GLVs grown in the crude effluent were cross sectioned, stained with Giemsa and safranin and microscopically viewed.

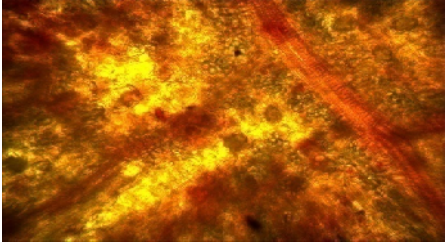
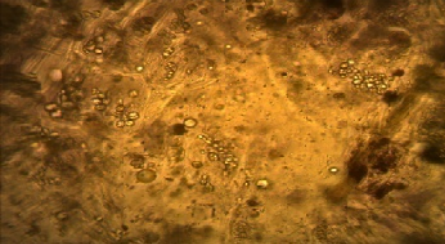
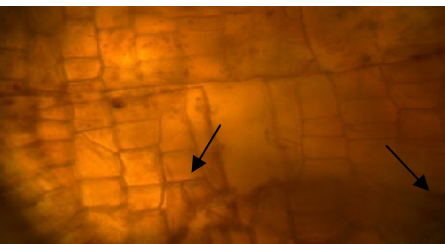
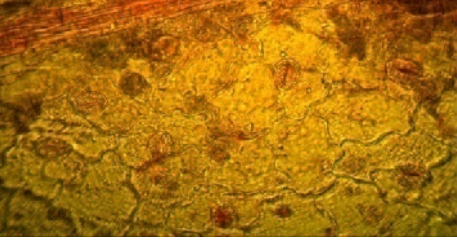

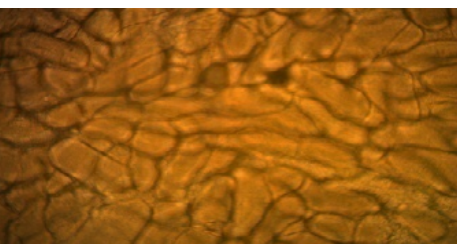
Plate 14 depicts the histological studies of the leaf, root and stem tissues of GLVs grown in crude effluent.

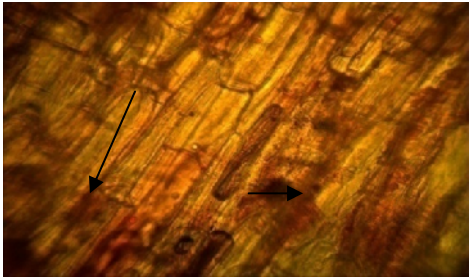
Plate 14

Histological studies of GLVs grown in crude effluent

<i>B.juncea</i>	Histological view	Observation
Leaf		A blurred appearance was viewed in the cells of the leaf
Root		The distorted roots were observed
Stem		Cross section of the stem cells showing cytoplasmic death of parenchymal cells

<i>T.foenum</i>	Histological view	Observation
Leaf		The chlorophyll was viewed but the plasmodesmata which acts as a channel was disconnected
Root		The root was not viewed clearly
Stem		The compartment of each cell was not viewed clearly may be due to the interaction of dye
Note: <i>T.foenum</i> plant did not survive on the 45 th day of 100% effluent, so 75% effluent exposed <i>T.foenum</i> were taken for histological analysis		
<i>A.polygonoides</i>	Histological view	Observation
Leaf		The leaf has chlorophyll in the edges with some black spots of dye interactions inside the cell
Root		The root viewed was partially clear with small granules
Stem		The stem cells were viewed within the granules throughout

<i>A.tristis</i>	Histological view	Observation
Leaf		An unclear view was observed.
Root		An indistinct view was observed
Stem		Cross section of the stem showing pith with dead cells in the corner
<i>S.grandiflora</i>	Histological view	Observation
Leaf		Few chloroplasts were observed
Root		The root was not clearly observed
Root nodules		The root nodules with compressed cells were observed

Stem		The parenchymal cells were viewed with irregular structural integrity and dead cells
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In Plate 14, the microscopic transverse section of the root and the stem viewed in all the GLVs were histologically observed and recorded. A clear view was not revealed in the root and the stem of the GLVs exposed by the effluent. These cytological abnormalities may be due to the presence of higher concentrations of the chemicals in the effluent that may disturb the normal cell cycle (Shobha, 2004). Clumpiness was revealed in the histological view of the root and the stem of the GLVs affected by the effluent.

Phase 3

Some microorganisms such as bacteria can degrade a wide range of dyes in the effluent. The biofertilizers (microbes) are effective, relatively cheaper, and has the ability to fix nitrogen, increase the plant growth, improve the soil fertility and to accelerate the degradation or decomposition of organic residues (Robinson *et al.*, 2001). The silk dyeing effluent treated by randomly selected biofertilizers (microbes) such as *Rhizobium sp.*, *Pseudomonas fluorescens* and *Azospirillum sp.*, of which the highest percentage decolorization was found in *Pseudomonas fluorescens* and *Azospirillum sp.*, both these biotreated effluent were selected for physico-chemical analysis. The biofertilizer *Pseudomonas fluorescens* maximally reduces the physico-chemical parameters of silk dyeing effluent was selected for the growth of the five selected GLVs. The post harvested stage of the GLVs on the 45th day was subjected to biometric parameters, phytochemical analysis, proximate principles, mineral contents, antioxidants and pigment analysis. The histological studies of the leaf, the stem and the root of GLVs grown in biotreated effluent and physico-chemical analysis of the biotreated effluent soil were also carried out.

4.3 Growth studies of the selected plants of GLVs grown in biotreated effluent

4.3.1 Physico-chemical analysis of the biotreated silk dyeing effluent

As percentage decolorization in different effluent concentrations by *Rhizobium sp.* was not remarkable and was not included for further studies.

4.3.1.1 Physical examination of the biotreated silk dyeing effluent

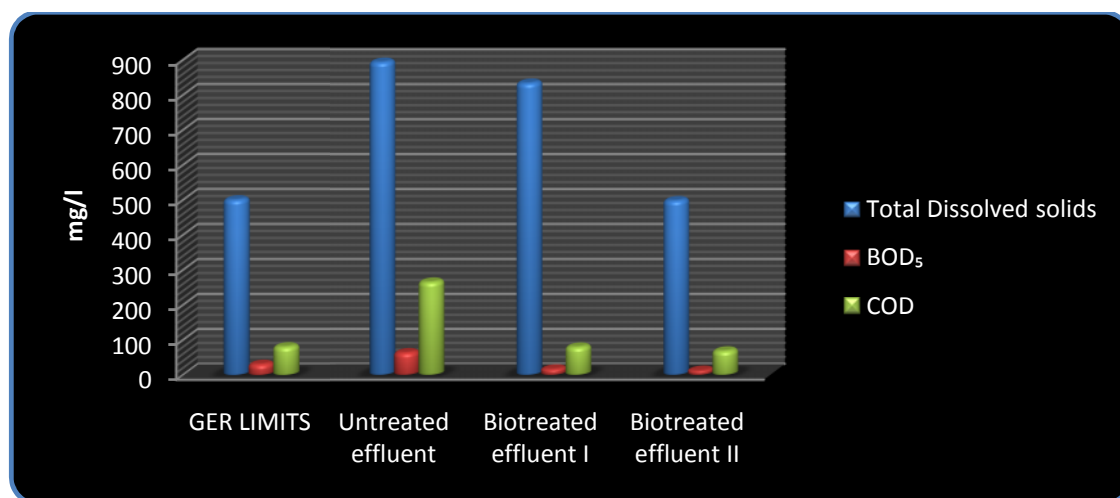
The appearance and color of the effluent after biotreatment I by *Azospirillum sp.* and biotreatment II by *Pseudomonas fluorescens* were slightly pinkish with slight turbidity which reflected the turbidity NT units reduced to 15 by *Azospirillum sp.* (biotreatment I) and maximally reduced to 5 by *Pseudomonas fluorescens* (biotreatment II).

The collected effluent did not exhibit any odour or foam. The electrical conductivity was also reduced maximally by *Pseudomonas fluorescens*.

The physical parameters of silk dyeing effluent of untreated and biotreated with *Azospirillum sp.*, and *Pseudomonas fluorescens* were revealed pictorially in Figure 4. The maximum reduction of TDS concentration was found in biotreatment II (498mg/l) which lies within the acceptable limits (500mg/l). Biological treatment process can be employed to remove substantial amount of BOD (Les, 2006). Among the two microorganisms used, *Pseudomonas fluorescens* (biotreatment II) reduced the BOD and COD to a maximum level.

Figure 4

Physical characterisation of the silk dyeing effluent in untreated and biotreated effluent



4.3.1.2 Chemical examination of the biotreated effluent

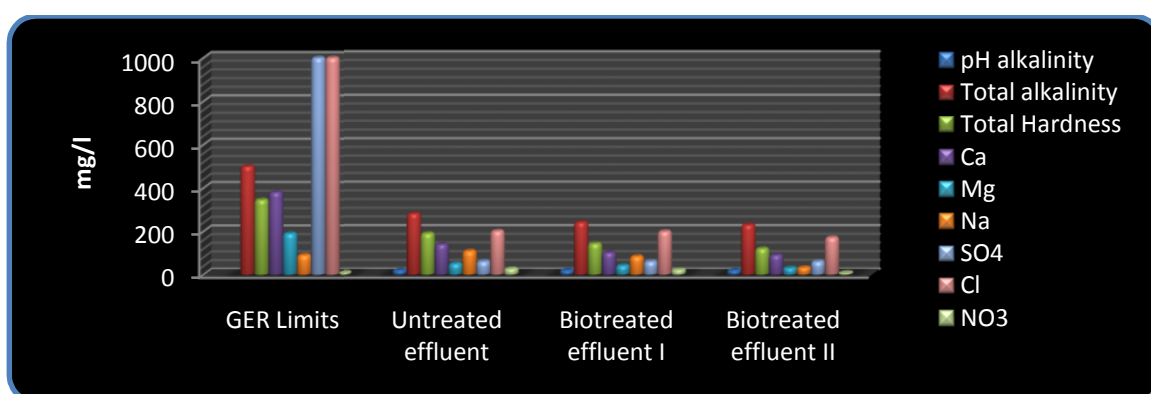
The pH range was maintained between 6 and 9. The biotreated effluent by *Azospirillum sp.* and *Pseudomonas fluorescens* were basic in nature. Though the GER limits (2010) is 37°C the collected effluent from the spot area (45°C) was reduced to room temperature 27°C. The biotreatment with *Azospirillum sp.* as well as *Pseudomonas fluorescens* did not change the pH alkalinity as CaCO₃ (20mg/l). The total hardness as CaCO₃ (which is calculated by the sum of Ca and Mg ion concentration levels) was reduced by both *Azospirillum sp.* and *Pseudomonas fluorescens*.

The levels of total hardness, calcium, magnesium, iron, free ammonia, chloride, fluoride, sulphate and phosphate of silk dyeing effluent were within the prescribed limits before biotreatment and were reduced maximally after biotreatment with *Pseudomonas fluorescens*. The manganese level was not detected in both untreated and biotreated samples. The sodium and nitrate of the effluent were above the set limits which were highly reduced by biotreatment II. Similar reduction was also detected for potassium and nitrite.

The Chemical characterization of untreated and biotreated silk dyeing effluent with *Azospirillum sp.* and *Pseudomonas fluorescens* were represented in Figure 5a, 5b and 5c respectively.

Figure 5a

Chemical characterisation of the untreated and biotreated silk dyeing effluent



The phosphate level was increased in both the biotreated effluents compared to the untreated effluents and was within the range. This might be due to the plant

growth promoting bacteria which shows a significant phosphatase activity and increased the phosphorus content as a result of mineralization of organic phosphates (Gyanshewar *et al.*, 2002).

Figure 5b

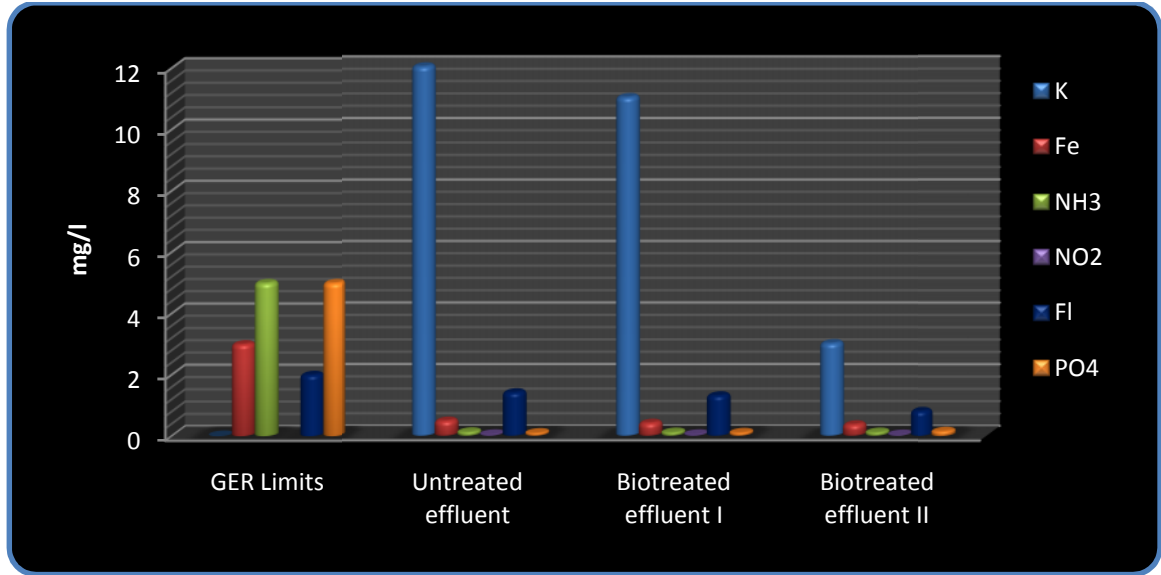
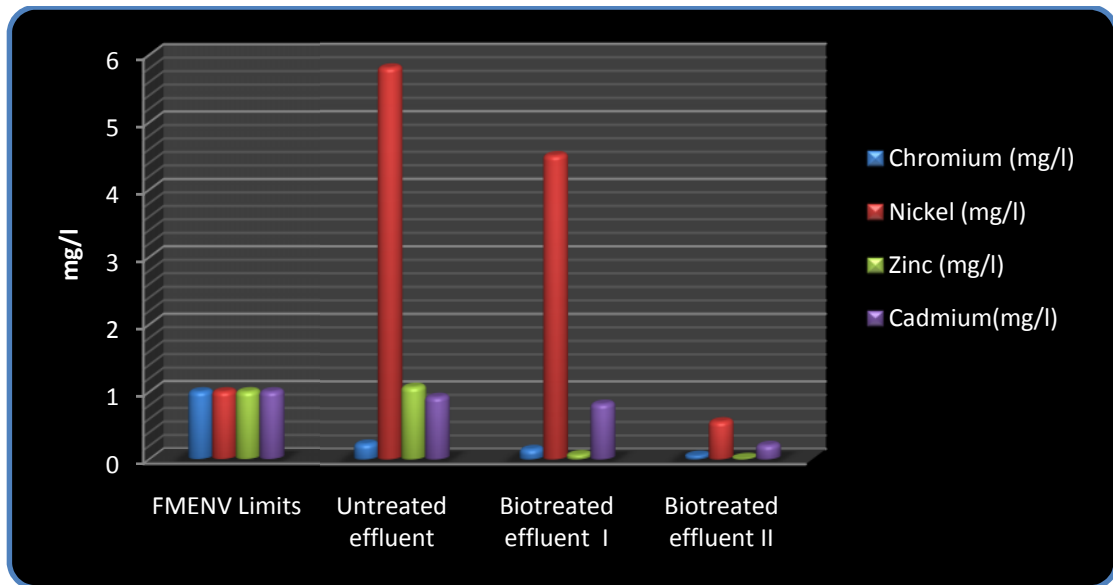


Figure 5c



The results revealed that the *Pseudomonas fluorescens* had efficiently reduced the physico-chemical characteristics of the silk dyeing effluent when compared to the *Azospirillum sp.*

In Figure 5c states that the heavy metals of silk dyeing effluent such as chromium, zinc, cadmium were within the FMENV limits, 1998. The nickel in the effluent was above the set limit which was highly reduced by biotreatment II. Lead was not detected in the effluent.

A study by Lu *et al.* (2011) also stated that the bioaugmentation process with the soil microorganism was capable of pollution degradation. The microorganisms are responsible for the degradation of heavy metals (Subhathra *et al.*, 2013). Thus from the present study, the unsafe silk dyeing effluent with chemical contaminants and heavy metals has been highly reduced. Among the two species, the *Pseudomonas fluorescens* maximally reduced the heavy metals, hence it was selected for further study.

4.3.1.3. Bioassay test

The bioassay test in fresh water and biotreated silk dyeing effluent by *Pseudomonas fluorescens* was represented in Table 6 and Plate 15.

Table 6
Bioassay test in fresh water and biotreated effluent

Fish exposed	No. of Individuals exposed	Percentage mortality						
		0h	12h	24h	48h	72h	96h	%
Fresh water (Serve as control) (from Table 10)	6	-	-	-	-	-	-	0
Biotreated silk dyeing effluent (with <i>Pseudomonas fluorescens</i>)	6	-	-	-	-	-	-	0

The bioassay test with 6 individual fish exposed to the fresh water serves as the control (Table 10) and was observed till 96 hrs and evaluated to be 0% mortality.

Analogous to it, the third batch of fish when experimentally observed also shows 0 % mortality when constantly checked from 0 hr - 96 hrs in the biotreated silk dyeing effluent water by *Pseudomonas fluorescens*. Laboratory studies had revealed the ability of

the bacteria in the removal of chemicals and heavy metals from industrial effluent (Sharmila *et al.*, 2013). Thus biotreated effluent supports the survival of the fish.

Plate 15

Bioassay test in fresh water and biotreated effluent



B 1



B 2



B 3



B 4



B 5



B 6



B 7



B 8



B 9

B 1 - 9 Fish grown in the biotreated silk dyeing effluent water by *Pseudomonas fluorescens* observed from 0-96 hrs. The pictorial representation confirms the 0% mortality. The fish grown in fresh water was shown in plate 4 (N 1-9)

4.3.2 Biometric parameters of the selected GLVs grown in biotreated effluent

4.3.2 Biometric parameters of the selected GLVs grown in biotreated effluent

PGPR (rhizo-biofertilizers) are a group of bacteria that actively colonize plant roots and enhance plant growth and yield (Jay, 2013). The potential of rhizosphere colonization by PGPB is very crucial and known as soil biofertilization (Villacieros *et al.*, 2003). These bacteria can improve the plant growth through various mechanisms have been known for decades and have been introduced into the soil, the seeds or the roots to improve the plant growth and health (Raaijmakers *et al.*, 2002). Bioinoculation increased the plant growth, leaf area, chlorophyll content, nodule numbers, nodule dry weight, pod number and dry weight, plant tissues and soil nutrient depletion (Anandaraj and Delapierre, 2010). Among the major groups of plant growth promoting bacteria, the most widely studied and efficient group include *Pseudomonas sp.* (Amy and Germida, 2002). The analysis of the physico-chemical characteristics as well as the bioassay test of silk dyeing effluent treated with *Pseudomonas fluorescens* had shown better results.

The biometric parameters were analysed on the 45th day of growth. Plate 16 illustrates the growth of the GLVs grown in biotreated effluent on the 45th day.

It indicates that the first cotyledon emerges within 2 days in *B.juncea* (BJT), *T.foenum* (TFT) and *A.polygonoides* (APT) and 3 days in *A.tristis* (ATT) and *S.grandiflora* (SGT) which were comparable to the GLVs grown in fresh water with normal condition. The percentage seed germination was maximum in *A.polygonoides* and *T.foenum* which were followed by *B.juncea*, *S.grandiflora* and *A.tristis* respectively. Subashini *et al.* (2007) reported that the biofertilizers can improve the plant growth by increasing the rate of seed germination.

Among the GLVs, *S.grandiflora* had recorded the maximum seedling length and *T.foenum* had shown the minimum and the findings were comparable to their growth in fresh water. Bhalla *et al.* (2007) also observed a maximum seedling length in *Dianthus carphyllus* grown in biofertilized soil. Among the GLVs, *A.tristis* and *S.grandiflora* recorded the highest number of leaves which was followed by *B.juncea* and *T.foenum*, whereas the *A.polygonoides* had shown the minimum number of leaves. This

reveals that the biotreated water supports a healthy environment for the growth of the GLVs.

Plate 16

Green leafy vegetables grown in biotreated effluent



BJT- *Brassica juncea* on the 45th day



TFT- *Trigonella foenum* on the 45th day



APT- *Amaranthus polygonoides* on the 45th day



ATT – *Amaranthus tristis* on the 45th day

SGT – *Sesbania grandiflora* on the 45th day

As percentage seed germination and seedling length were high in *B.juncea* and *S.grandiflora* similar to the GLVs grown in fresh water. This study was in accordance with the study of Warade *et al.* (2007) that the treatment of biofertilizer significantly increased the plant height and number of leaves in Dahlia.

The surface area of leaf was found to be high in *B.juncea* which was similar to the results shown by *B.juncea* grown in fresh water. Among the selected GLVs *S.grandiflora* and *B.juncea* were shown the maximum shoot length; the other GLVs recorded a comparable shoot length. Mia *et al.* (2010) showed that the effect of PGPR inoculation resulted in maximum shoot length growth compared to the uninoculated control in *Musa* plant. The root length as well as the root shoot ratio was found to be maximum for *A.polygonoides* and *S.grandiflora* when compared to the other GLVs. The study by Ramalakshmi and Raj, (2008) had reported a significant increase in root length of cotton plant grown in biofertilized soil. The maximum root mass was recorded in *S.grandiflora* which was followed by *B.juncea* and was similar to the results shown by their growth in fresh water.

The percentage dry matter was found to be comparable for all the GLVs except *T.foenum* with the least dry matter as its seedling length was also measured to be less compared to the other GLVs. This study correlates with the results of Saniz *et al.* (2004) that the amendment of soil with biofertilizers significantly increased the dry matter yields of red clover and cucumber plant. The number of seed bunch per plant was maximum in *B.juncea* and minimum in *T.foenum* whereas numerous seed bunch was seen in *A.polygonoides* and *A.tristis*. The *S.grandiflora* seeds were not found in the experimental period. The distance between nodes was found to be maximum in *S.grandiflora*, *A.tristis* and *A.polygonoides* and minimum in *B.juncea* and *T.foenum*.

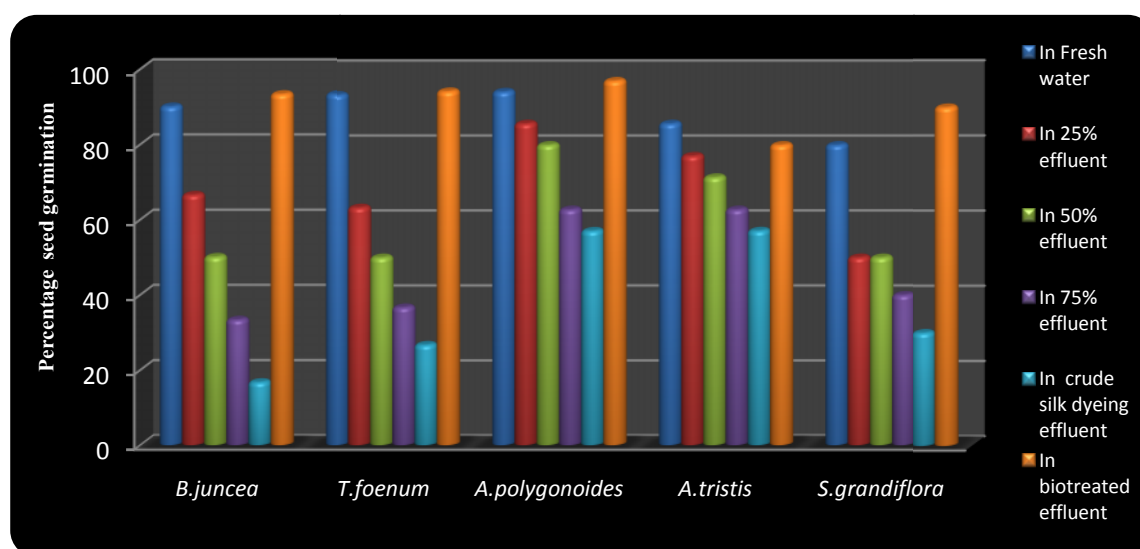
The results of our study correlates with the findings of Gholami *et al.* (2009) that the PGPR and *Pseudomonas fluorescens* significantly enhanced seed germination, shoot dry weight, leaf surface area, plant height and number of seed in maize. Ribaudó *et al.* (2006) observed that the inoculation of biofertilizers in the soil promoted the development of tomato plant with increased shoot, root dry weight, shoot height and total root surface area.

The inoculation of plant growth promoting rhizobacteria (PGPR) significantly increased the shoot length, the root length and the dry matter production of shoot and root of *Cicer arietinum* (Mishra *et al.*, 2010).

4.3.2.1 Effect of fresh water, different concentrations of effluent and biotreated effluent on seed germination of the selected GLVs

The percentage seed germination of the selected GLVs treated with fresh water, different concentrations of the effluent and biotreated effluent was presented in Figure 6.

Figure 6
Seed germination of the selected GLVs



The percentage seed germination of all the GLVs treated with different concentrations of silk dyeing effluent was found to be very low when compared to their germination in fresh water and biotreated effluent. Also the percentage of seed germination declined with the increasing concentrations of the effluent. Whereas the percentage seed germination of all the GLVs in fresh water and biotreated effluent was nearing or above 90%. So it is clear that both the treatments (fresh water and biotreated effluent supported the germination of GLVs to the maximum.

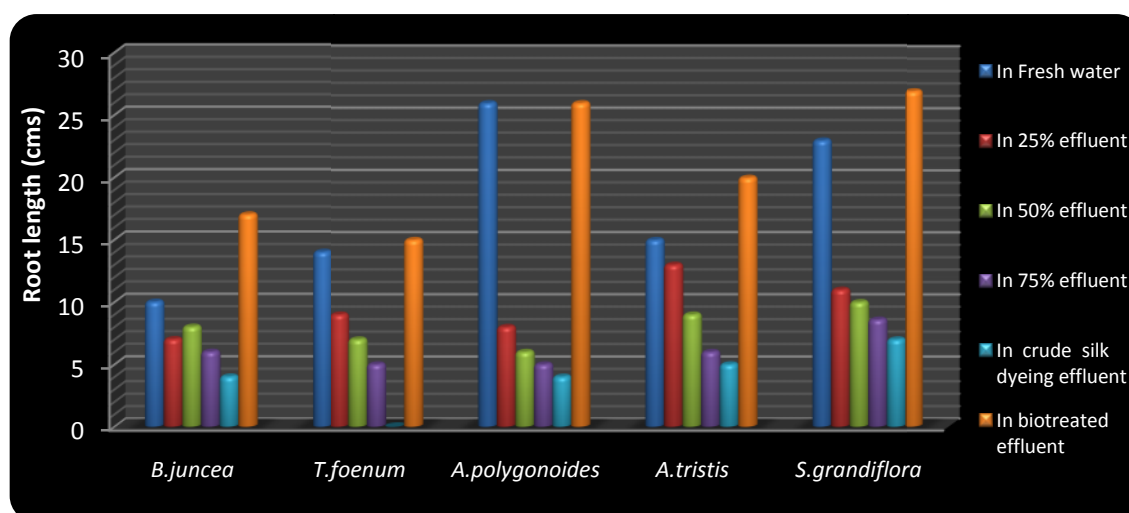
4.3.2.2 The root length of the selected GLVs grown in fresh water, different concentrations of the effluent and biotreated effluent

The root length of the GLVs grown in fresh water, 25%-100% effluent and biotreated effluent was illustrated in Figure 7.

The root length of all the plants was affected significantly by the treatment with crude silk dyeing effluent. The maximum reduction in the root length of the selected GLVs was noticed in 100% effluent. The increasing concentrations of the effluent gradually decrease the average root length. The biotreated GLVs except *T.foenum* and *A.polygonoides* enhanced the root length to the maximum when compared to the GLVs grown in fresh water.

Figure 7

Root length of the selected GLVs in different treatments



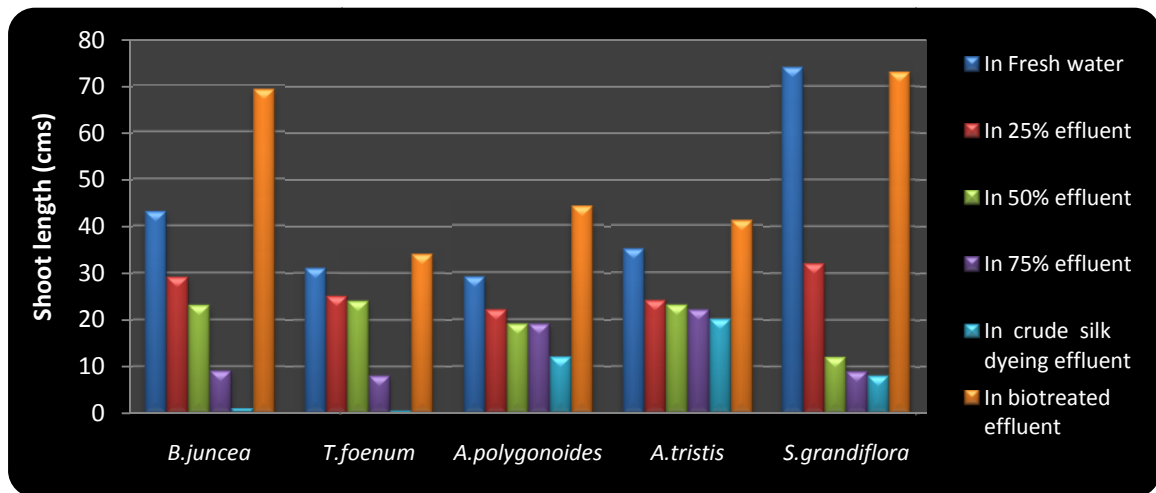
Thus it was clear that the *Pseudomonas fluorescens* treated effluent was found to be effective when compared to the different concentrations of the effluent. Similar finding was found by Tharakeshwari and Shobha (2006) in which root length was reduced in 100% distillery effluent when compared to the control plant of *Vigna radiata*.

4.3.2.3 Influence of fresh water, different concentrations of effluent and biotreated effluent on the shoot length of the selected GLVs

Figure 8 explains the difference in shoot length of the GLVs subjected to various treatments.

Figure 8

Shoot length of the selected GLVs as influenced by various treatments



The significant difference in shoot length was noticed in control, effluent exposed and biotreated GLVs. There was a significant increase in the shoot length of *T.foenum*, *A.polygonoides* and *A.tristis* grown in biotreated effluent when compared to fresh water. Whereas the shoot length of the *B.juncea* and *S.grandiflora* did not differ very much between fresh water and biotreated effluent. The increase in effluent concentration was inversely proportional to the shoot length.

4.3.3 Qualitative phytochemical parameters of the selected GLVs grown in biotreated effluent

The biotreated effluent was used to grow the selected GLV till 45th day. The leaf, the stem and the seed of the selected plants (except the seed of *S.grandiflora* as the seeds were produced after the experimental period) after the post harvested stage were removed, air dried and powdered separately using a blender and methanolic extracts of the same were prepared.

Table 7 indicates the phytochemical constituents of the methanolic extract of the leaves, the stems and the seeds of the selected green leafy vegetables grown in biotreated effluent.

Table 7

Qualitative analysis of the phytochemical constituents of the selected GLVs grown in biotreated effluent

Name of the Nutrient	<i>B.juncea</i>			<i>T.foenum</i>			<i>A.polygonoides</i>			<i>A.tristis</i>			<i>S.grandiflora</i>	
	Leaf	Stem	Seed	Leaf	Stem	Seed	Leaf	Stem	Seed	Leaf	Stem	Seed	Leaf	Stem
Carbohydrate	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Proteins	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenol	+	+	+	+	-	+	Tr	Tr	-	-	+	+	+	+
Catechol	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sterols	+	+	Tr	+	+	Tr	+	+	Tr	Tr	Tr	Tr	+	+
Glycosides	-	+	+	-	+	+	-	-	+	+	+	+	-	-
Saponin	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Quinones	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cynogenic glycosides	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	-	+	Tr
Flavonoids	-	+	+	-	-	-	-	-	-	Tr	+	Tr	-	+
Leucoanthocyanidines	-	Tr	-	-	-	-	-	-	-	Tr	-	+	-	Tr
Tannins	+	+	+	+	+	+	-	-	Tr	+	+	-	+	-
Anthocyanins	+	+	+	+	+	+	Tr	Tr	Tr	+	+	+	+	+
Volatile oils	-	+	-	-	-	-	-	-	-	-	Tr	-	-	-
Lignin	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Terpenoids	+	+	+	-	+	+	+	-	+	Tr	-	+	+	+
Cellulose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Free aminoacids	-	+	+	+	+	+	+	+	+	-	+	-	-	+
Starch	-	-	-	-	-	+	-	+	+	-	-	+	-	-
Reducing sugars	+	+	+	-	-	+	-	-	+	-	-	+	-	+

+ Presence - Absence Tr – Trace presence

The prominent presence of carbohydrate, protein, cellulose and quinone in the leaves, the stems and the seeds of the methanolic extract of the biotreated GLVs except *S.grandiflora* which was similar to fresh water treatment. The cyanogenic glycosides, catechol, saponin and lignin were completely absent in all parts of the selected GLVs. The phenol was present prominently in all the parts of *B.juncea*, in the leaf and the seeds of *T.foenum* and in the stem and the seed of *A.tristis*. The sterol is prominently present in the leaf and the stem of *B.juncea*, *T.foenum*, *A.polygonoides* and *S.grandiflora* plants and also slightly present in the leaf and the stem of *A.tristis* and all the seeds of the GLVs. The glycoside was absent in all the leaf extracts of the GLVs except *A.tristis*. The stem and the seeds of *B.juncea*, *T.foenum* and the seed of *A.polygonoides* indicated the prominent presence of glycoside. The alkaloid was present in all parts of the *B.juncea*, *T.foenum* and *A.polygonoides* except the seeds of the *A.tristis*. The complete absence of flavonoid was noticed in all parts of *T.foenum* and *A.polygonoides* and the leaves of *B.juncea* and *S.grandiflora* and is present in the stem and the seeds of *B.juncea* and the stem of *A.tristis* and *S.grandiflora* plants. The slight presence of flavonoids was observed in the leaf and the seeds of *A.tristis*.

The leucoanthocyanidine was absent in all parts of the *T.foenum* and *A.polygonoides* and present in the seeds of *A.tristis* and slight presence in the stem of *B.juncea* and *S.grandiflora* and in the leaf of *A.tristis*. The tannin was present in all parts of the *B.juncea* and *T.foenum*. The leaf, the stem of *A.polygonoides*, the seed of *A.tristis* and the stem of *S.grandiflora* indicates its absence. The volatile oil was absent in all parts of the GLVs except the stem of *B.juncea* and *A.tristis*. The terpenoid was present in all the GLVs except the leaves of *T.foenum* and stem of *A.polygonoides* and *A.tristis*. The free aminoacids were present in all parts of all the GLVs except the leaf of *B.juncea*, *A.tristis* and *S.grandiflora* and the leaf and the seed of *A.tristis*. The starch was absent in all parts of the GLVs except the seeds of *T.foenum*, *A.tristis* and in the stem and the seed of *A.polygonoides*. The reducing sugar was prominently present in all parts of *B.juncea* and the seeds of *T.foenum*, *A.polygonoides* and *A.tristis* plants. The phytochemical constituents of the GLVs grown in fresh water and biotreated effluent did not differ significantly. Thus the biotreated effluent did not affect the phytochemical constituents of the GLVs.

4.3.4 Proximate principles and mineral contents of the selected GLVs grown in biotreated effluent

The moisture content of all the selected GLVs ranges between 15 and 30%. The maximum moisture content was recorded by *A.tristis*, *B.juncea* and *A.polygonoides* and minimum by *T.foenum* and *S.grandiflora*. Among the GLVs, *A.tristis* recorded significantly ($p<0.05$) high moisture content.

Comparable ash content was recorded by all the selected GLVs except *A.polygonoides* with maximum value ($p<0.05$). A recent study by Clement in 2011 had reported similar moisture content in *Cleome gynandra*. The percentage crude fiber of *S.grandiflora* and *A.tristis* was similar and maximum whereas the other three GLVs had shown a minimum and comparable crude fiber. Similar crude fiber content was recorded in Basidiocarps of *Lentinus squarrosulus* by Krishnendu *et al.*, in 2013. A significantly higher ($p<0.05$) total carbohydrate was found in *S.grandiflora* and minimum in *B.juncea*. A study by Lim and Rabeta, (2013) in milk apple had shown similar carbohydrate content as in *B.juncea*. The maximum level of protein was detected in *S.grandiflora* and minimum in *A.polygonoides* and *T.foenum*. The cellulose content showed approximately higher values in *B.juncea* and *S.grandiflora* which was followed by *A.tristis*, *A.polygonoides* and *T.foenum* respectively.

The highest iron content was noticed in *T.foenum* with a lowest value in *S.grandiflora* which was similar to the GLVs grown in fresh water. Similar protein, carbohydrate and iron content were recorded in *Solanum melongena* by Agoreyo *et al.* in 2012. The level of phosphorus of all the selected GLVs was found to be comparable except *S.grandiflora*. A study by Konieczynski and Wesolowski (2007) had reported a similar phosphorus level in *Betula pendula*. Velmurugan *et al.* (2007) also reported that the biofertilizer application enhanced the phosphorus in tumeric.

The calcium and magnesium were found to be maximum in *B.juncea* and minimum in *S.grandiflora*. The application of plant growth promoting rhizobacteria resulted in the highest iron and magnesium contents in the apple leaves (Karasurt and Aslantas, 2010).

Thus the proximate principles and mineral contents were found to be higher in biotreated effluent when compared to the GLVs grown in fresh water and untreated effluent water.

4.3.4.1 Effect of fresh water, effluent and biotreated effluent on proximate principles of the selected GLVs

The influence of fresh water, untreated effluent and biotreated effluent on the proximate principles such as moisture content, ash content, crude fibre, total carbohydrate, total protein and cellulose in the selected GLVs were depicted in Figure 9, 10 and 11 respectively.

Leafy green vegetables are an important component of the human diet, providing fibre, minerals and vitamins (Acikgoz, 2011). The moisture content of the GLVs grown in untreated effluent was found to be higher than the GLVs grown in fresh water and biotreated effluent. This is due to the decrease in dry weight of the GLVs in untreated effluent. Among the GLVs, *A.tristis* plant grown in effluent shows the highest moisture content which was followed by *S.grandiflora* and *A.polygonoides* respectively. The GLVs grown in various treatments had shown a comparable ash content. The crude fibre of all the GLVs differs significantly with the maximum for the biotreated *S.grandiflora*. There was a significant change in the cellulose content of the GLVs exposed to different treatments.

Figure 9

Moisture content of the selected GLVs in different treatments

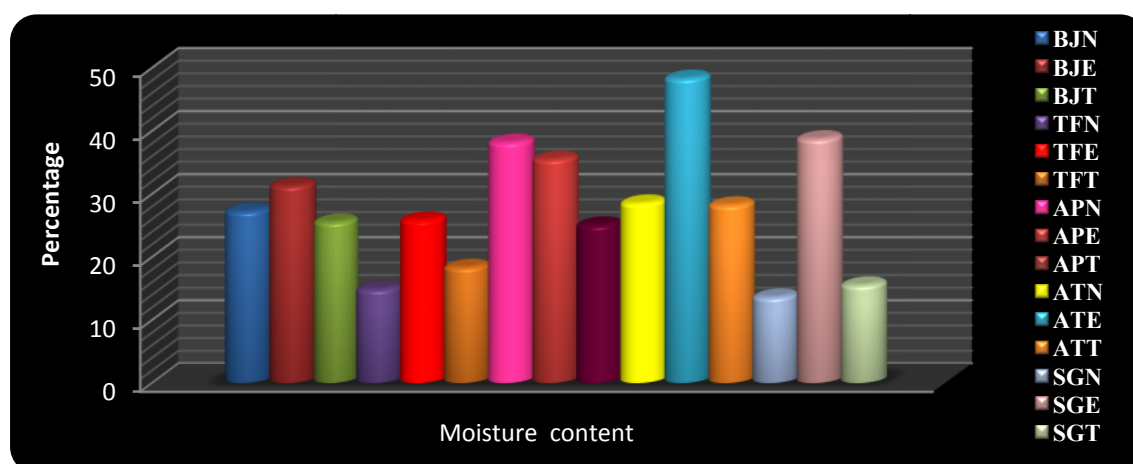
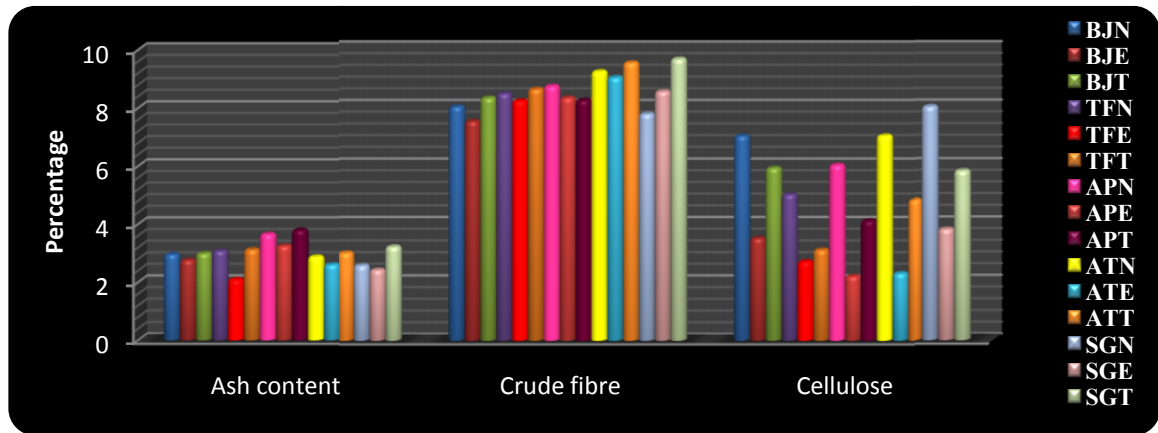
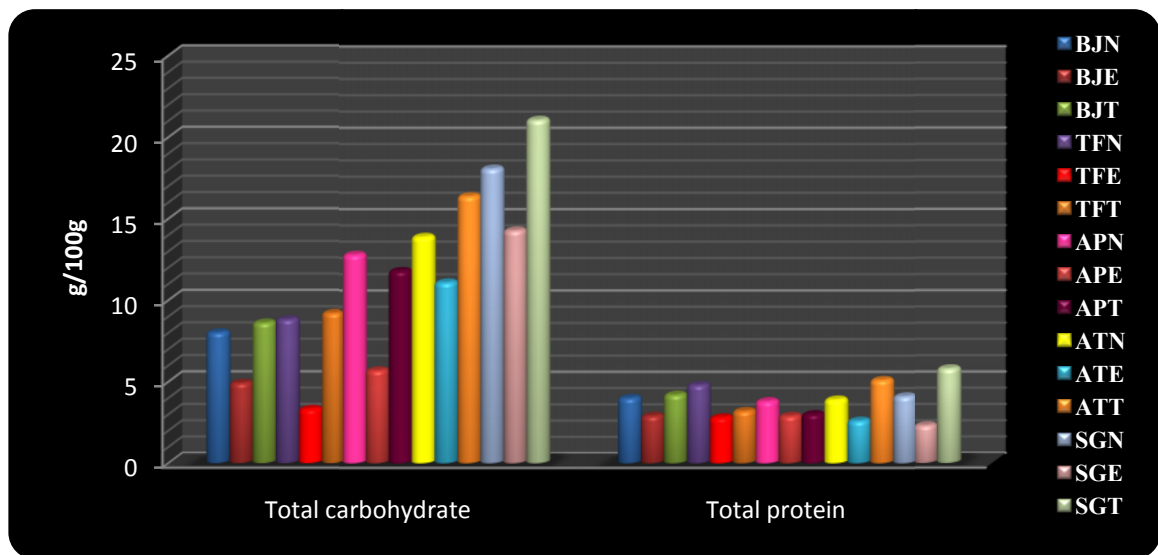


Figure 10
Ash content, crude fibre and cellulose of the selected GLVs in different treatments



BJN: *Brassica juncea*, TFN: *Trigonella foenum*, APN: *Amaranthus polygonoides*, ATN: *Amaranthus tristis*, SGN: *Sesbania grandiflora* were grown in fresh water.
 BJE: *Brassica juncea*, TFE: *Trigonella foenum*, APE: *Amaranthus polygonoides*, ATE: *Amaranthus tristis*, SGE: *Sesbania grandiflora* were grown in 75% effluent water.
 BJT: *Brassica juncea*, TFT: *Trigonella foenum*, APT: *Amaranthus polygonoides*, ATT: *Amaranthus tristis*, SGT: *Sesbania grandiflora* were grown in biotreated effluent.

Figure 11
Total carbohydrate and Total protein of the selected GLVs in different treatments



BJN: *Brassica juncea*, TFN: *Trigonella foenum*, APN: *Amaranthus polygonoides*, ATN: *Amaranthus tristis*, SGN: *Sesbania grandiflora* were grown in fresh water.
 BJE: *Brassica juncea*, TFE: *Trigonella foenum*, APE: *Amaranthus polygonoides*, ATE: *Amaranthus tristis*, SGE: *Sesbania grandiflora* were grown in 75% effluent water.
 BJT: *Brassica juncea*, TFT: *Trigonella foenum*, APT: *Amaranthus polygonoides*, ATT: *Amaranthus tristis*, SGT: *Sesbania grandiflora* were grown in biotreated effluent.

The carbohydrate content of the biotreated *S.grandiflora* plant was comparately higher than the other GLVs and the minimum recorded by *T.foenum* grown in effluent. This shows that the effluent has strong influence on the *T.foenum* followed by *B.juncea*. The other GLVs grown in effluent manage to produce carbohydrate above 5% level. The protein level was found to be maximum in the biotreated *S.grandiflora* and minimum in the biotreated *A.polygonoides* when compared to other GLVs.

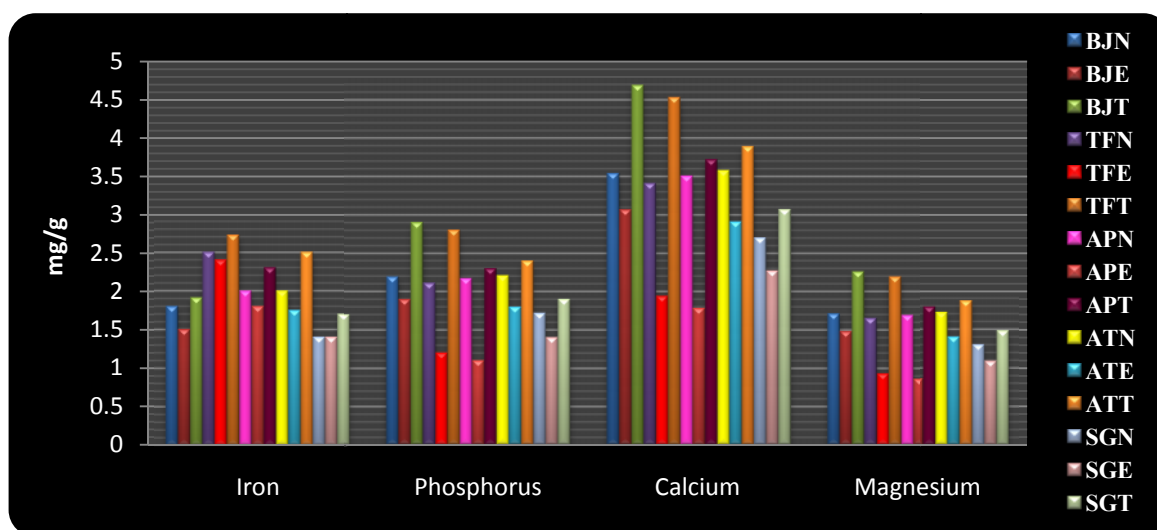
Thus increase in proximate principles of the GLVs clearly indicates that the biotreated effluent (*Pseudomonas fluorescens*) supports the growth of the GLV plants.

4.3.4.2 Influence of fresh water, silk dyeing effluent and biotreated effluent on the mineral contents of the selected GLVs

Figure 12 represents the comparison of the iron, phosphorus, calcium and magnesium content of the selected GLVs exposed to different treatments.

Figure 12

Mineral contents of the selected GLVs in different treatments



BJN: *Brassica juncea*, TFN: *Trigonella foenum*, APN: *Amaranthus polygonoides*, ATN: *Amaranthus tristis*, SGN: *Sesbania grandiflora* were grown in fresh water.

BJE: *Brassica juncea*, TFE: *Trigonella foenum*, APE: *Amaranthus polygonoides*, ATE: *Amaranthus tristis*, SGE: *Sesbania grandiflora* were grown in 75% effluent water.

BJT: *Brassica juncea*, TFT: *Trigonella foenum*, APT: *Amaranthus polygonoides*, ATT: *Amaranthus tristis*, SGT: *Sesbania grandiflora* were grown in biotreated effluent.

The minerals are the important constituents that are required in minimum levels for plants. There was a significant change in the mineral composition of the GLVs grown in various treatments. The iron content of the biotreated plants was found to be maximum in *T.foenum* and *A.tristis*. The effluent decreases the iron level of the GLVs when compared to fresh water. The iron content of *B.juncea* was decreased to a greater extent when compared to other GLVs. Nevertheless the iron level of *S.grandiflora* grown in effluent doesn't get affected. Enhanced iron nutrition resulting in increased plant growth can also be achieved due to the ability of some plants to bind and release iron from bacterial iron-siderophore complexes and utilizing the iron for growth (Bashan and de-bashan, 2005). The phosphorus level was increased in biotreated plants in the order of *B.juncea*>*T.foenum*>*A.tristis*>*A.polygonoides*> *S.grandiflora*. The phosphorus level was maximally reduced in *A.polygonoides* and *T.foenum* grown in the effluent. The calcium and magnesium were highly affected in the *T.foenum* and *A.polygonoides* compared to the other GLVs grown in effluent. The calcium and magnesium of the GLVs grown in biotreated effluent were found to be maximum when compared to both the untreated and effluent treated plants.

Thus it was clear that the GLVs grown in biotreated effluent (*Pseudomonas fluorescens*) were found to be a good source of minerals.

4.3.5 Enzymic, Non enzymic antioxidants and Lipid peroxidation of the selected GLVs grown in biotreated effluent

The activity of SOD of all GLVs ranges between 1.00- 3.10 U/g and the highest activity was reported in *A.tristis* and *S.grandiflora* and the lowest activity in *B.juncea*. A comparative study by Karthikeyan *et al.* (2007) reported that the application of biofertilizers resulted in increased SOD activity in the *Catharanthus roseus* plant. A significantly higher ($p<0.05$) peroxidase activity was recorded by *S.grandiflora* which was followed by *A.tristis* and *B.juncea* whereas the *T.foenum* and *A.polygonoides* were shown the minimum peroxidase activity. A similar peroxidase activity was reported in *Cephalandra indica* by Vijayakumari *et al.* (2012). A study by Mahmood (2010) also reported that the application of Rhizobacteria significantly increased the peroxidase activity in the leaves of banana plants.

Among the GLVs, *A.polygonoides* had shown the maximum phenolic content and the *T.foenum* with the minimum phenolic content. A study by Khaled *et al.* (2007) had also shown the lowest phenolic content in *Adonis palaestina Boiss* and the highest in *Salvia fruticosa*. A similar study by Vijayakumari and Janardhanan, (2003) reported that the maximum phenolic content was found in silk cotton by the application of biofertilizers. The *T.foenum* was found to be superior with the highest ascorbic acid content and was found to be significant at 5% level. This was followed by *S.grandiflora*. The *B.juncea* has the least ascorbic acid content. The *A.polygonoides* and *A.tristis* had shown a comparable ascorbic acid content.

A corresponding study by Sudhakar and Purushutham, (2008) showed that the application of biofertilizers significantly increased the ascorbic acid content in *Solanum lycopersicum*. The tocopherol content in *T.foenum*, *A.tristis* and *A.polygonoides* were comparable and that of *S.grandiflora* and *B.juncea* were identical. The vitamin E (Tocopherol) and vitamin C (Ascorbic acid) are the potent free radical scavengers and prevent the oxidative damage by utilizing the free radicals (Antony *et al.*, 2003).

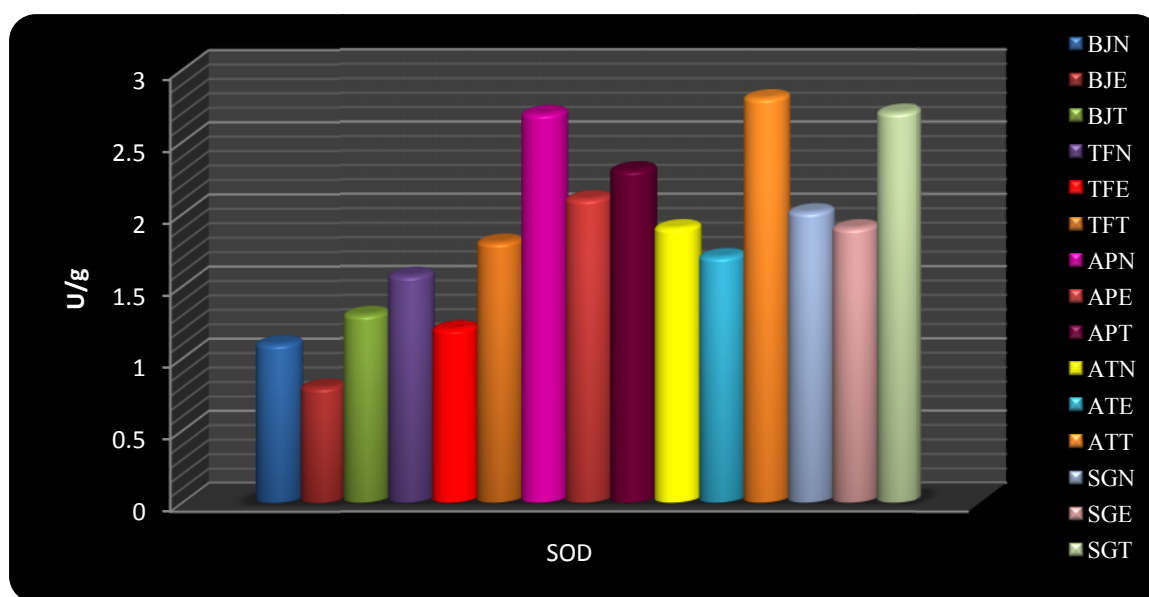
The free radical scavenging activity of H₂O₂ was in the range of 61-93%. *S.grandiflora* had shown a significantly ($p < 0.05$) H₂O₂ scavenging activity which was followed by *B.juncea* and *A.tristis*. The *T.foenum* and *A.polygonoides* had shown similar H₂O₂ scavenging activity. *S.grandiflora* was also found to be superior with maximum DPPH activity followed by *A.tristis* and *A.polygonoides* with similar activity. The *B.juncea* and *T.foenum* had shown the minimum and comparable DPPH activity. The results of this study were comparable to the study by Kubola *et al.* (2011) with similar DPPH activity in *Flacourtia indica*. Thus the green leafy vegetables grown in biotreated effluent provide a good source of antioxidant activity which in turn reflects that the GLVs were grown in a healthy environment. This was supported by the study of Shailu and Ramesh, (2011) that the enzymic antioxidants were improved in *Brassica juncea* plants grown in bioremediated effluent.

4.3.5.1 Influence of fresh water, silk dyeing effluent and biotreated effluent on the enzymic antioxidant and lipid peroxidation activity of the selected GLVs

The enzymic antioxidant activity such as SOD and peroxidase and lipid peroxidation such as H₂O₂ scavenging activity and DPPH activity of the selected GLVs grown in various treatments were depicted graphically in Figure 13, 14 and 15 respectively.

Figure 13

Superoxide dismutase activity of the selected GLVs in different treatments



BJN: *Brassica juncea*, TFN: *Trigonella foenum*, APN: *Amaranthus polygonoides*, ATN: *Amaranthus tristis*, SGN: *Sesbania grandiflora* were grown in fresh water.

BJE: *Brassica juncea*, TFE: *Trigonella foenum*, APE: *Amaranthus polygonoides*, ATE: *Amaranthus tristis*, SGE: *Sesbania grandiflora* were grown in 75% effluent water.

BJT: *Brassica juncea*, TFT: *Trigonella foenum*, APT: *Amaranthus polygonoides*, ATT: *Amaranthus tristis*, SGT: *Sesbania grandiflora* were grown in biotreated effluent.

Figure 14

Peroxidase activity of the selected GLVs in different treatments

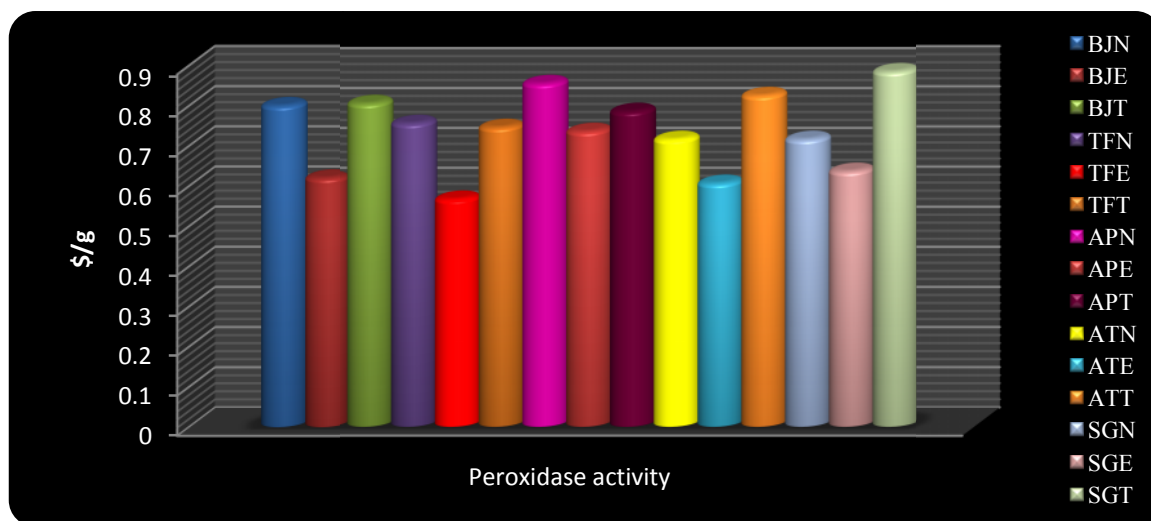
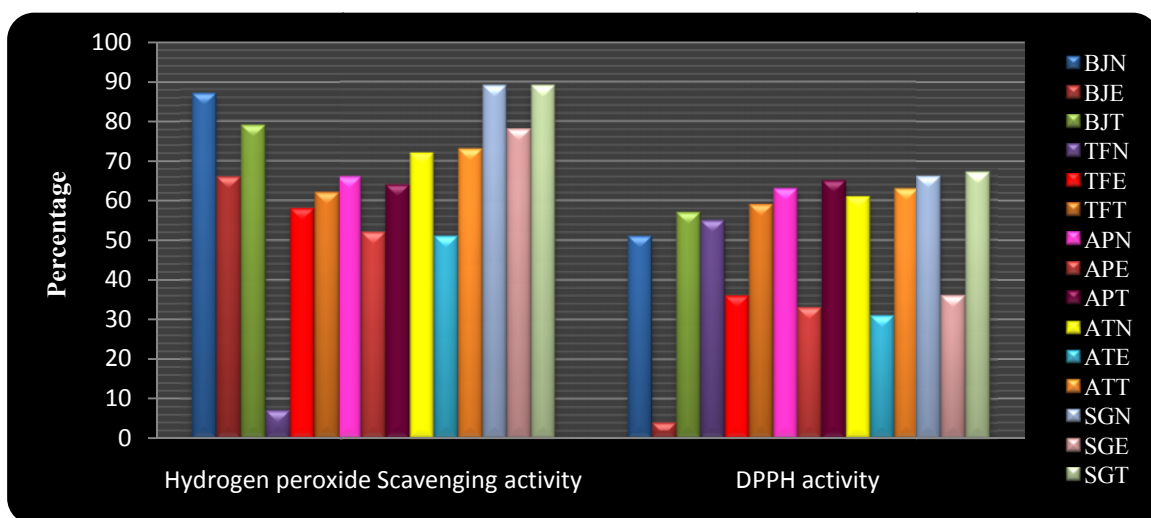


Figure 15

Hydrogen peroxide scavenging activity and DPPH activity of the selected GLVs in different treatments



^aU = Amount of enzyme that gives 50% inhibition of the extent of NBT reduction in 1 min

\$/unit – Change of absorbance / minute at 430 nm

BJN: *Brassica juncea*, TFN: *Trigonella foenum*, APN: *Amaranthus polygonoides*, ATN: *Amaranthus tristis*, SGN: *Sesbania grandiflora* were grown in fresh water.

BJE: *Brassica juncea*, TFE: *Trigonella foenum*, APE: *Amaranthus polygonoides*, ATE: *Amaranthus tristis*, SGE: *Sesbania grandiflora* were grown in 75% effluent water.

BJT: *Brassica juncea*, TFT: *Trigonella foenum*, APT: *Amaranthus polygonoides*, ATT: *Amaranthus tristis*, SGT: *Sesbania grandiflora* were grown in biotreated effluent.

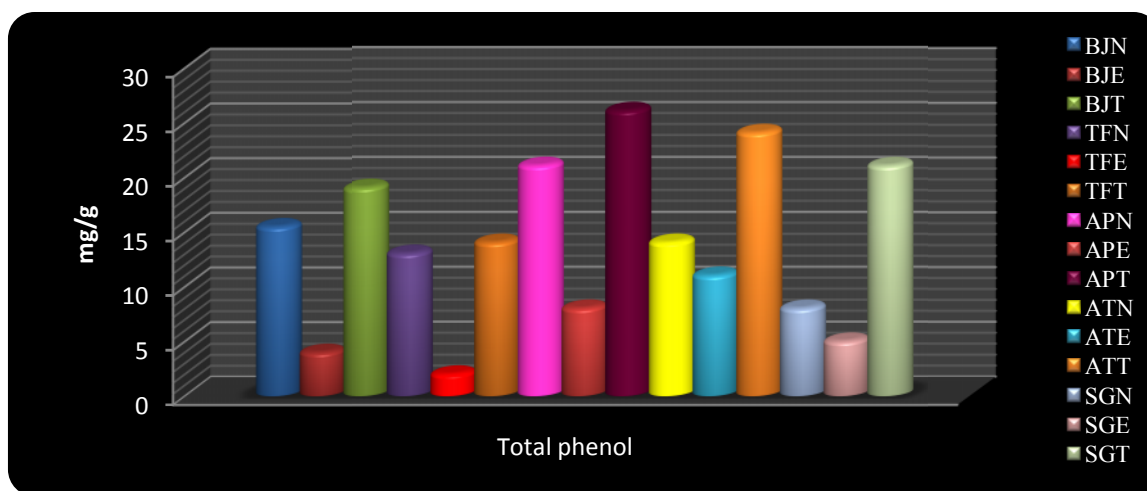
The SOD activity in all the GLVs except *A.polygonoides* was found to be maximum for biotreated effluent when compared to the plants grown in fresh water and untreated effluent. Among the GLVs the biotreated *A.tristis* and *S.grandiflora* were found to be superior with the highest SOD activity. A similar study by Chaparzadeh *et al.* (2004) was in agreement with the above results that the application of biofertilizers influenced the SOD activity in *Calendula officinalis*. The GLVs grown in biotreated and fresh water had shown the higher peroxidase activity, H₂O₂ activity and DPPH activity when compared to the GLVs grown in crude effluent. Kina and Nikitina (2009) also stated that the application of biofertilizers increased the peroxidase activity in the roots of wheat seedlings. There was a significant difference in peroxidase activity as well as lipid peroxidation for all the GLVs grown in various treatments.

4.3.5.2 Effect of fresh water, 75% effluent and biotreated effluent on the non- enzymic antioxidants of the selected GLVs

Figure 16, 17 and 18 depict the non enzymic antioxidants such as total phenol, ascorbic acid and tocopherol respectively in the selected GLVs grown in different treatments.

Figure 16

Total phenol levels of the selected GLVs in different treatments



BJN: *Brassica juncea*, TFN: *Trigonella foenum*, APN: *Amaranthus polygonoides*, ATN: *Amaranthus tristis*, SGN: *Sesbania grandiflora* were grown in fresh water.

BJE: *Brassica juncea*, TFE: *Trigonella foenum*, APE: *Amaranthus polygonoides*, ATE: *Amaranthus tristis*, SGE: *Sesbania grandiflora* were grown in 75% effluent water.

BJT: *Brassica juncea*, TFT: *Trigonella foenum*, APT: *Amaranthus polygonoides*, ATT: *Amaranthus tristis*, SGT: *Sesbania grandiflora* were grown in biotreated effluent.

Figure 17

Ascorbic acid levels of the selected GLVs in different treatments

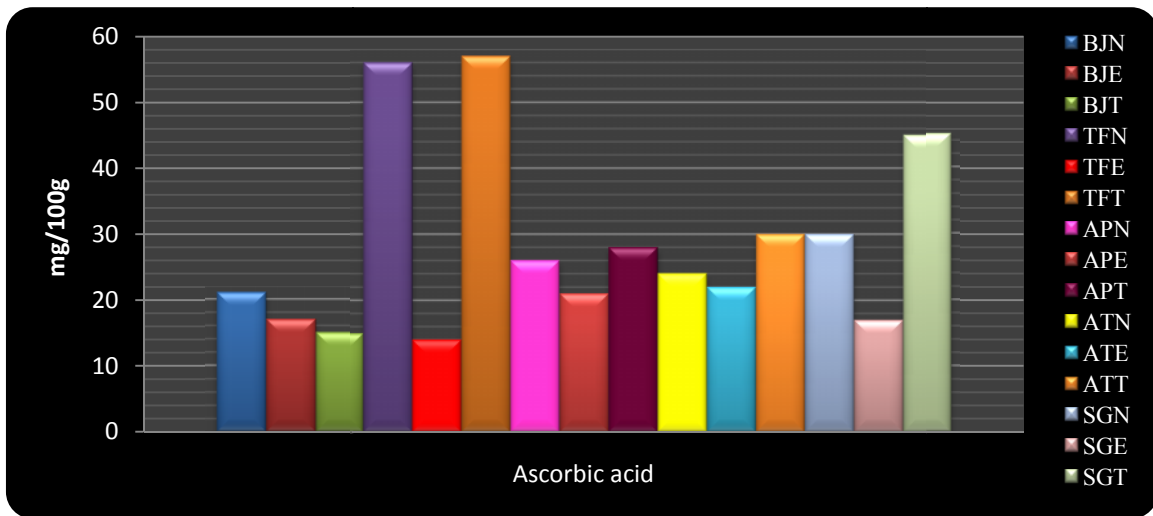
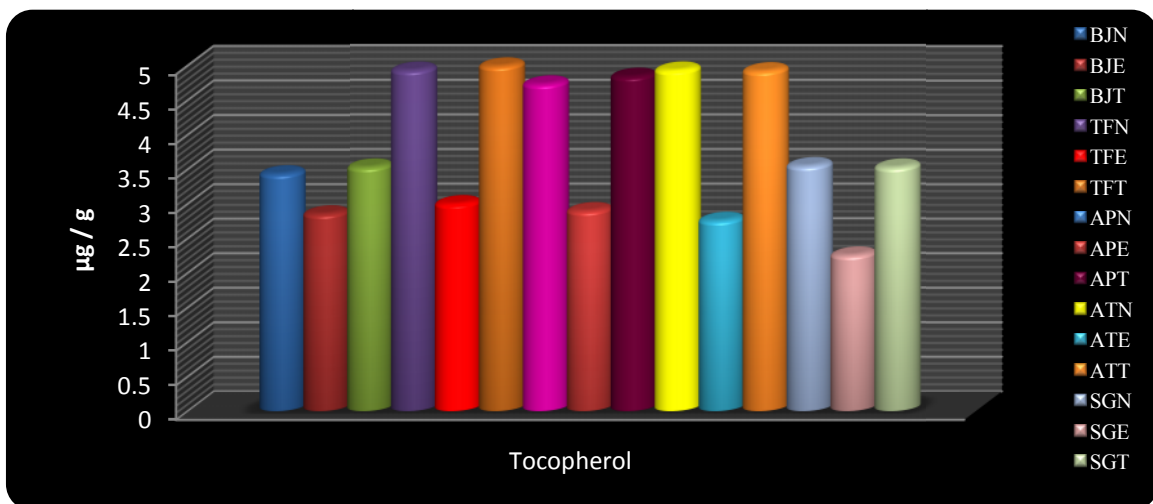


Figure 18

Tocopherol levels of the selected GLVs in different treatments



BJN: *Brassica juncea*, TFN: *Trigonella foenum*, APN: *Amaranthus polygonoides*, ATN: *Amaranthus tristis*, SGN: *Sesbania grandiflora* were grown in fresh water.

BJE: *Brassica juncea*, TFE: *Trigonella foenum*, APE: *Amaranthus polygonoides*, ATE: *Amaranthus tristis*, SGE: *Sesbania grandiflora* were grown in 75% effluent water.

BJT: *Brassica juncea*, TFT: *Trigonella foenum*, APT: *Amaranthus polygonoides*, ATT: *Amaranthus tristis*, SGT: *Sesbania grandiflora* were grown in biotreated effluent.

The total phenol content was significantly high in *A.polygonoides*, *A.tristis* and *S.grandiflora* grown in biotreated effluent whereas it was highly reduced in *T.foenum*

followed by *B.juncea* and *S.grandiflora* grown in untreated effluent. The tocopherol level of each of the GLVs did not vary much between fresh water and biotreated effluent. The vitamin C level of the *T.foenum* grown in fresh water and biotreated effluent was maximum when compared to the other GLVs. The levels of ascorbic acid of all the GLVs grown in 75% effluent were reduced significantly.

4.3.6 Pigment levels of the selected GLVs grown in biotreated effluent

The total chlorophyll content was increased in the order of *A.tristis*<*A.polygonoides*<*T.foenum*<*B.juncea*<*S.grandiflora*. Among the GLVs, *S.grandiflora* recorded a significantly ($p<0.05$) maximum level of total chlorophyll. The carotenoid level was maximum in *B.juncea* which was followed by *S.grandiflora*, *T.foenum* and *A.polygonoides*. *A.tristis* recorded a minimum level of carotenoids.

Thus the GLVs grown in biotreated effluent soil has increased the chlorophyll and carotenoid levels, which in turn reveals that the biotreatment of the effluent with *Pseudomonas fluorescens* had degraded the toxic compounds present in the crude effluent. Velmurugan *et al.* (2007) also reported that the total chlorophyll content in cauliflower was maximum by the application of biofertilizers. A study by Upadhyay *et al.* (2007) had also shown the maximum total carotenoid content in cabbage (*Brassica oleracea*) grown in soil treated with biofertilizers.

4.3.6.1 Comparison of pigment levels of the selected GLV plants grown in fresh water, 75% effluent and biotreated effluent

The pigments such as total chlorophyll (Chl_a and Chl_b) and carotenoids levels subjected to different treatments were illustrated in Figure 19 and 20.

Figure 19

Total chlorophyll of the selected GLVs in different treatments

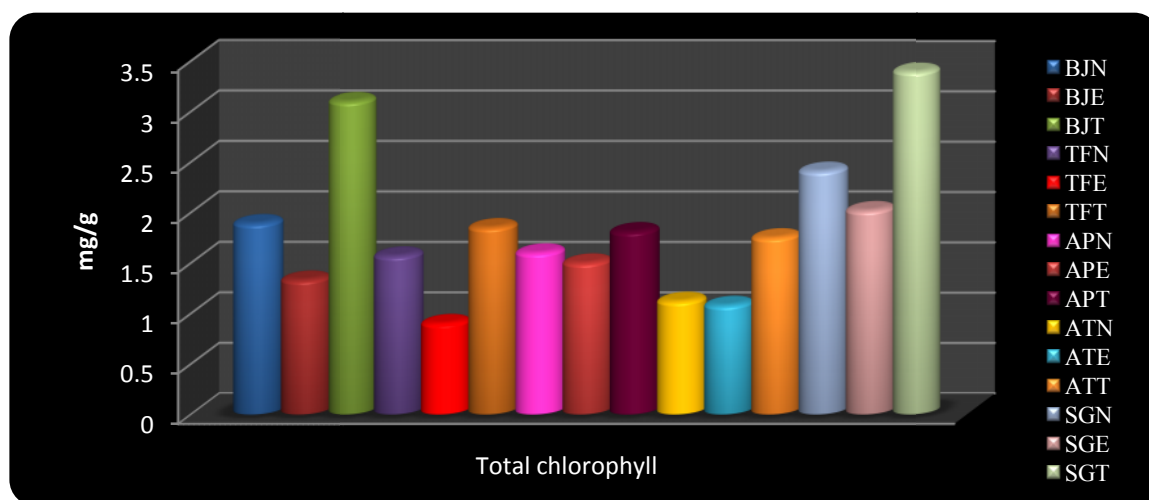
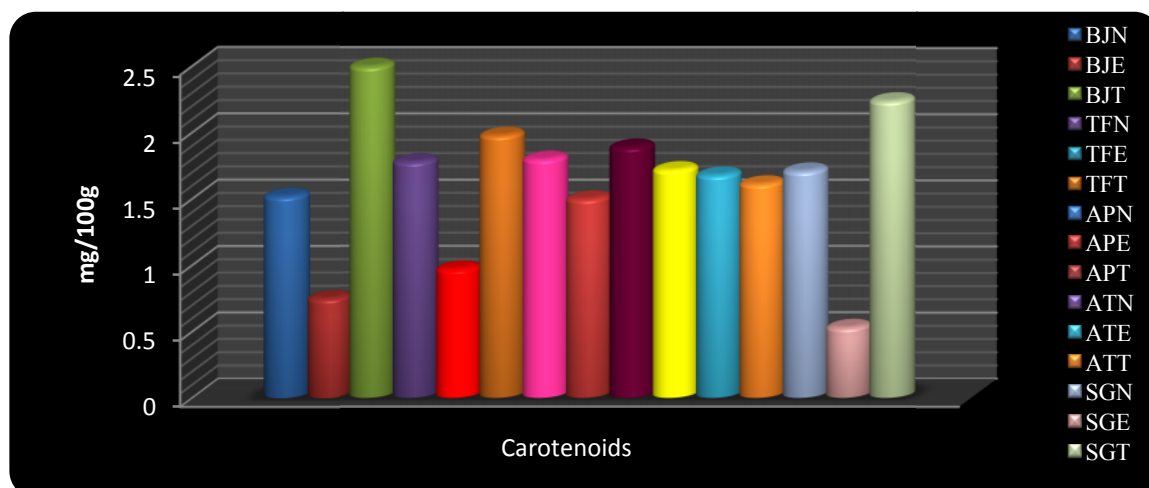


Figure 20

Carotenoids of the selected GLVs in different treatments



BJN: *Brassica juncea*, TFN: *Trigonella foenum*, APN: *Amaranthus polygonoides*, ATN: *Amaranthus tristis*, SGN: *Sesbania grandiflora* were grown in fresh water.

BJE: *Brassica juncea*, TFE: *Trigonella foenum*, APE: *Amaranthus polygonoides*, ATE: *Amaranthus tristis*, SGE: *Sesbania grandiflora* were grown in 75% effluent water.

BJT: *Brassica juncea*, TFT: *Trigonella foenum*, APT: *Amaranthus polygonoides*, ATT: *Amaranthus tristis*, SGT: *Sesbania grandiflora* were grown in biotreated effluent.

The results of the study showed that the total chlorophyll and carotenoids were significantly high in biotreated *S.grandiflora* and *B.juncea*. The total chlorophyll level was highly reduced in the *T.foenum* grown in 75% effluent. In case of carotenoids the most affected plant was *S.grandiflora* and the least affected was *A.tristis* grown in 75% effluent.

The biotreated *B.juncea* has an elevated level of carotenoids when compared to other GLVs grown in biotreated effluent. The results are in accordance with the findings of Selvarathi *et al.* in 2010 that the photosynthetic pigments such as Chl_a, Chl_b, total chlorophyll and carotenoid contents were increased in the plant *Lycopersium esculentum* grown in soil treated with biofertilizers.

4.3.7 Physico-chemical analysis of the biotreated effluent soil

Biofertilizers are the products containing living cells of different types of microorganisms that enrich the nutrient quality of the soil. The availability of nutrients for plants utilized from the soil is regulated by the rhizospheric microbial activity (Herrmann *et al.*, 2005).

Similar to the control, the pH and EC of biotreated soil were within the standard limits. The soil texture was same throughout the study. The results also indicated that the biotreatment improves the micronutrient status of the soil within the optimal range. It is due to the influence of biofertilizer in the biotreated soil that had improved the levels of Ca, N, P and K from 105, 76, 4, 137 ppm in the effluent treated soil to 110, 200, 14.5, 390 ppm respectively in the biotreated effluent soil. Das *et al.* (2009) observed that the application of biofertilizers improved the available N, P and K contents in the soil as well as in the plant. The micronutrients Fe, Mn, Cu and Zn ranges from 1.2, 10, 0.4, 0.2 ppm of effluent treated soil to 6.1, 26.22, 2.26, 2.13 ppm respectively in the biotreated effluent soil.

Thus it was clear that the impact of effluent on the soil nutrients was encountered by the biofertilizer.

4.3.7.1 Influence of fresh water, crude effluent and biotreated effluent on the macronutrients and micronutrient of the soil.

The macronutrients and micronutrients of the soil of different treatments were depicted in the Figure 21, 22, 23 and 24 respectively.

It was clear that the macronutrients such as calcium, nitrogen, phosphorus and potassium and the micronutrients such as iron, manganese, copper and zinc were improved in the biotreated effluent soil compared to the effluent contaminated soil.

Figure 21

Calcium in the soil of different treatments

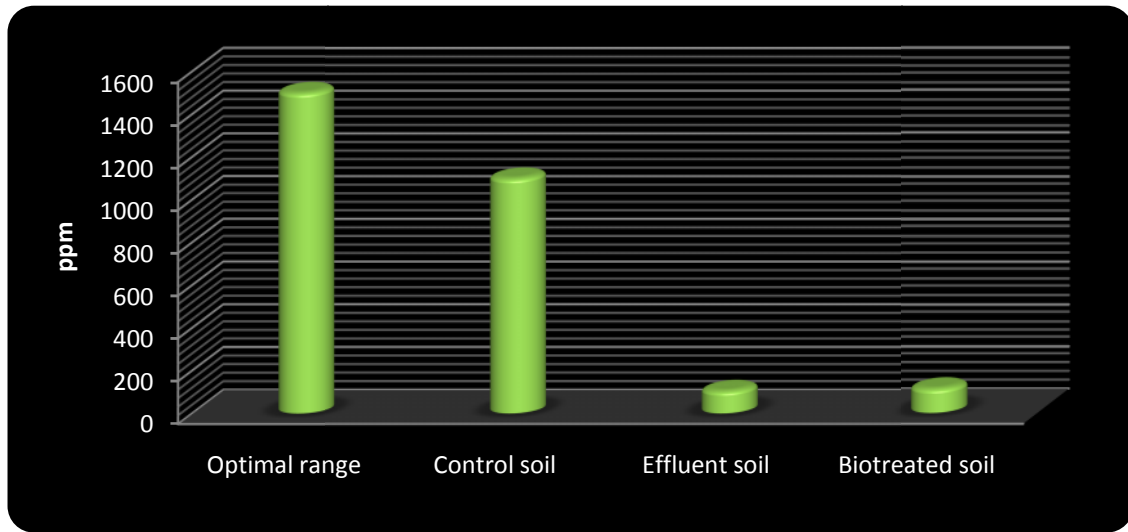


Figure 22

Nitrogen, phosphorus and potassium in the soil of different treatments

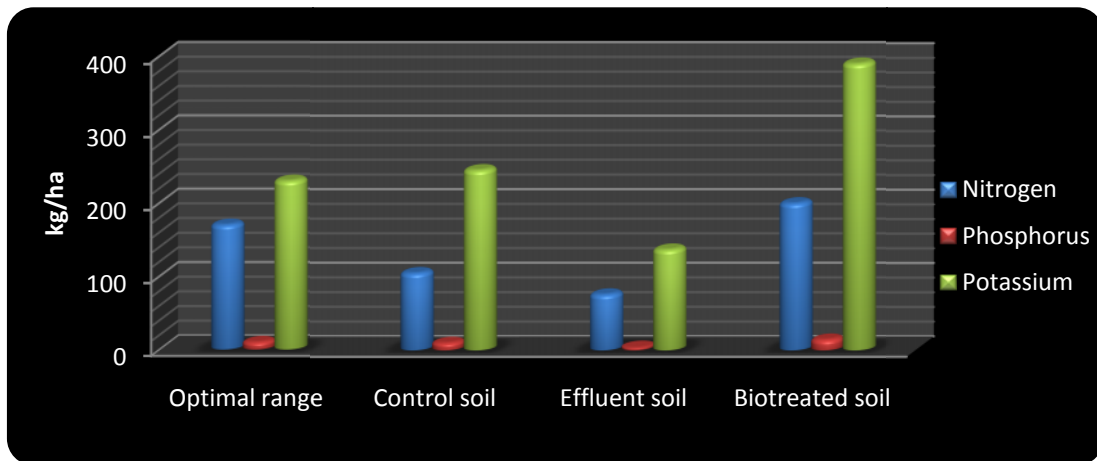


Figure 23

Iron and manganese in the soil of different treatments

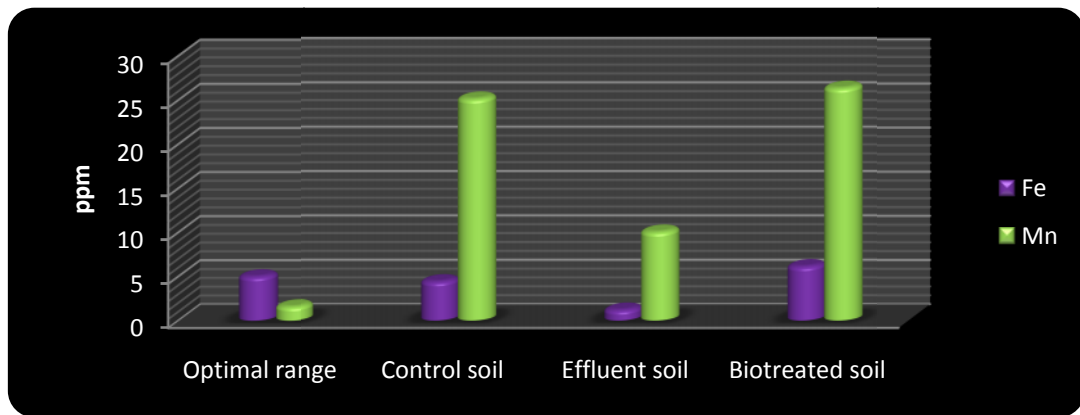
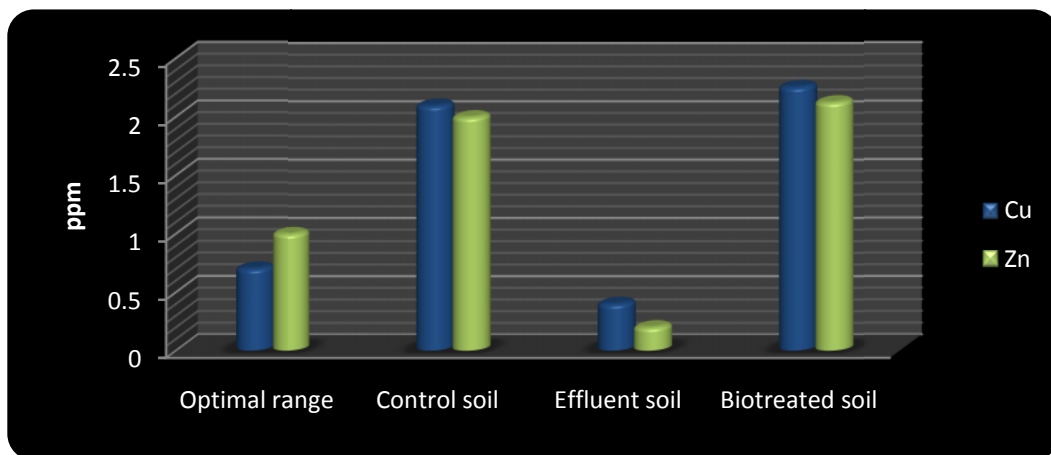


Figure 24

Copper and zinc in the soil of different treatments



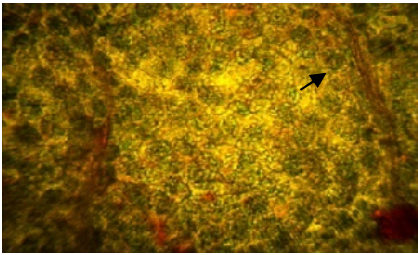
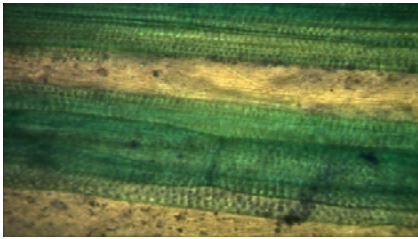
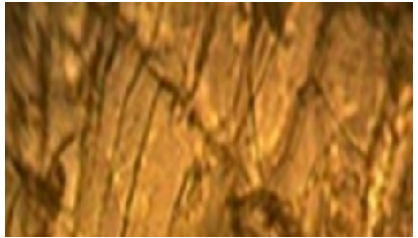
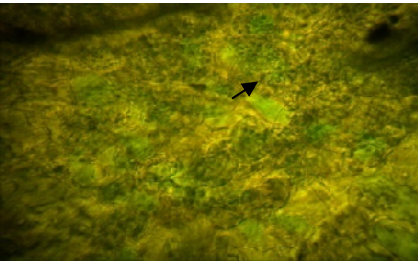
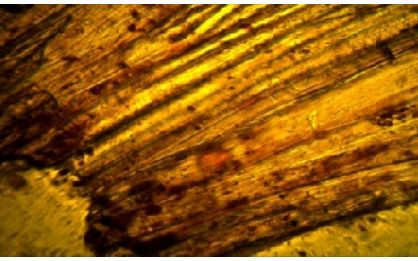
Thus it was clear from the present study that the biotreatment had improved the macro and micronutrients of the effluent contaminated soil and can be adapted as cost effective and ecofriendly to sustain the productivity of the green leafy vegetable crops

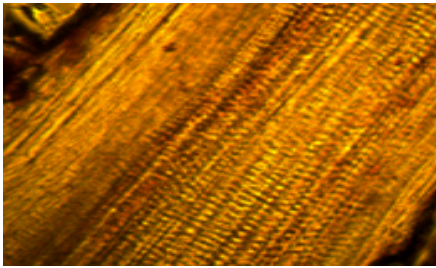
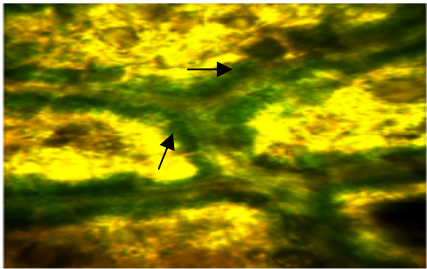
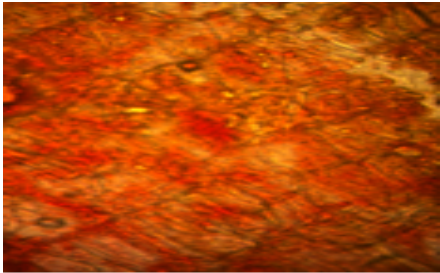
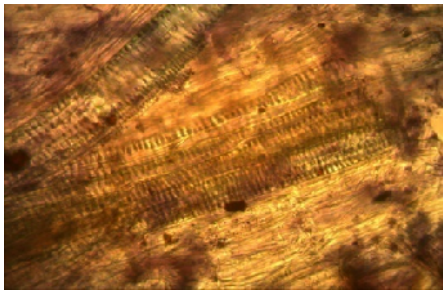
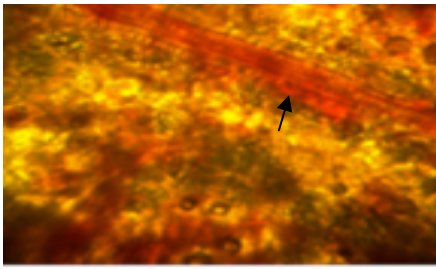
4.3.8 Histological studies of the selected plants of GLVs grown in biotreated effluent

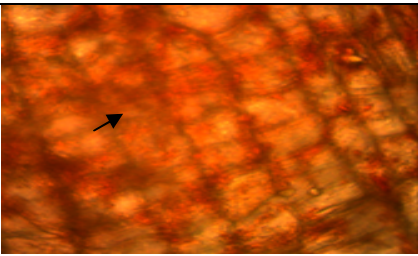
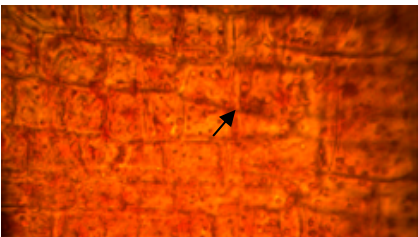
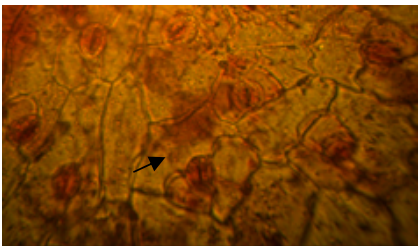
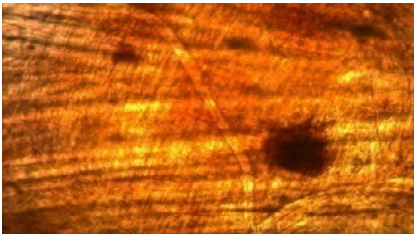
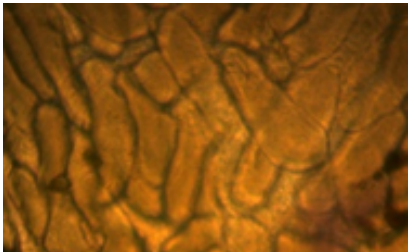
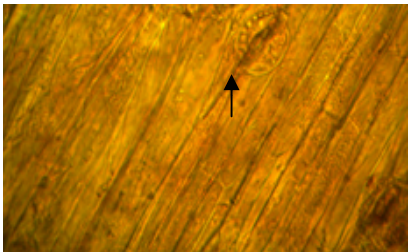
Plate 17 illustrates the histological studies of the leaf, the root and the stem tissues of GLVs grown in biotreated effluent.

Plate 17

Histological studies of GLVs grown in biotreated effluent

<i>B.juncea</i>	Histological view	Observation
Leaf		The clear cells visualized in the leaf of <i>B.juncea</i> with plasmadesmata
Root		The roots were observed clearly with the lipid bilayer
Stem		The stem cells were seen with segregated cells
<i>T.foenum</i>	Histological view	Observation
Leaf		The plenty of chloroplasts were observed which in turn produces the green color
<i>T.foenum</i>	Histological view	Observation
Root		The tangential section with intact cells in the roots were viewed with compartments

Stem		The lipid bilayer of the radial section of the stem with compartments was viewed
<i>A. polygonoides</i>	Histological view	Observation
Leaf		The chloroplast cells were seen at the corner
Root		The clear cells with granules were viewed
Stem		The stem cells were viewed with lipid bilayer
<i>A. tristis</i>	Histological view	Observation
Leaf		The chloroplast and plasmadesmata were viewed clearly

Root		Intact cells were observed
Stem		The cell compartments were viewed with the granules throughout
<i>S.grandiflora</i>	Histological view	Observation
Leaf		Chloroplasts were viewed within the intact cell
Root		The root hairs were seen clearly
Root nodules		The root nodules shows intact cells
Stem		The stem cells were viewed with stomata (which allows gaseous exchange for the processes of respiration and photosynthesis) in-between

The sections of the root, the stem and the leaf for all the GLVs grown in the biotreated effluent were histologically observed which were similar as that of the GLVs grown in fresh water.

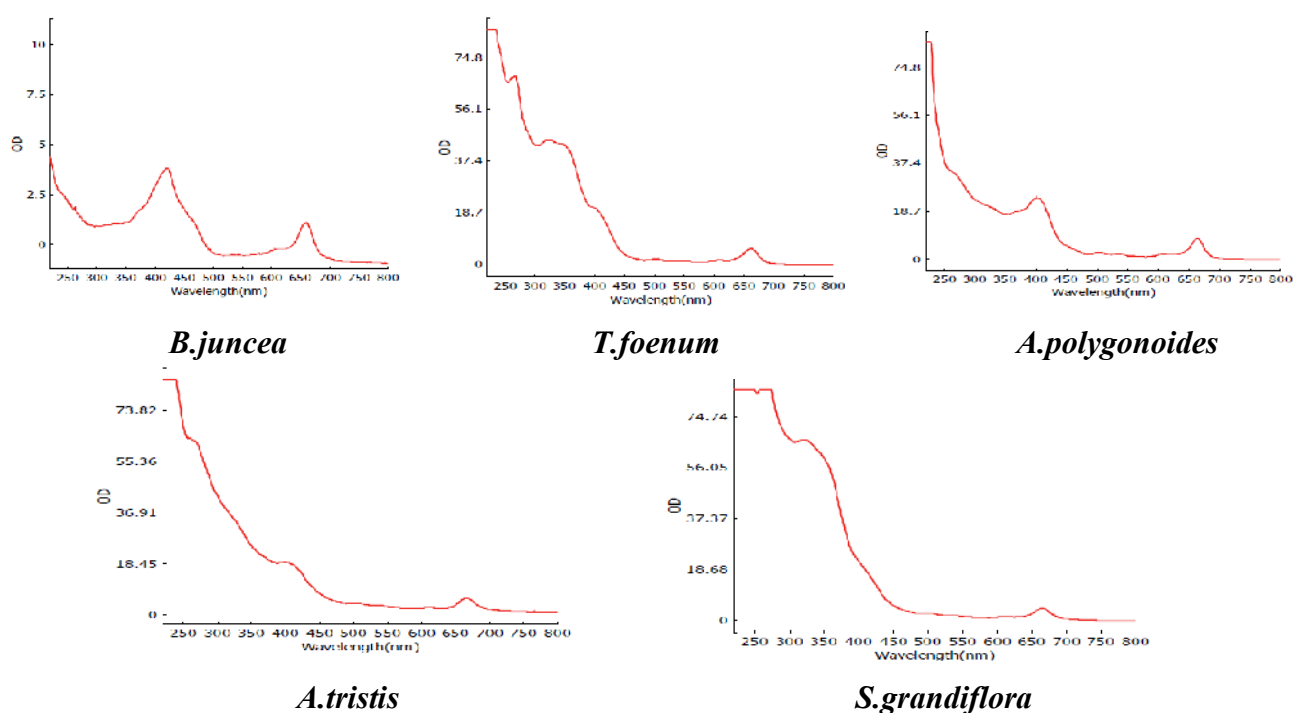
Phase 4

4.4. Identification of the functional groups and compounds in selected GLV plants using spectroscopic and chromatographic techniques

4.4.1. Analysis of the methanolic extracts of selected GLVs grown in fresh water in bionano UV visible spectrophotometer

Figure 25 depicts the spectra of methanolic extracts of five selected GLVs grown in fresh water subjected to bionano UV visible spectrophotometer.

Figure 25
UV visible spectra of the five selected GLVs



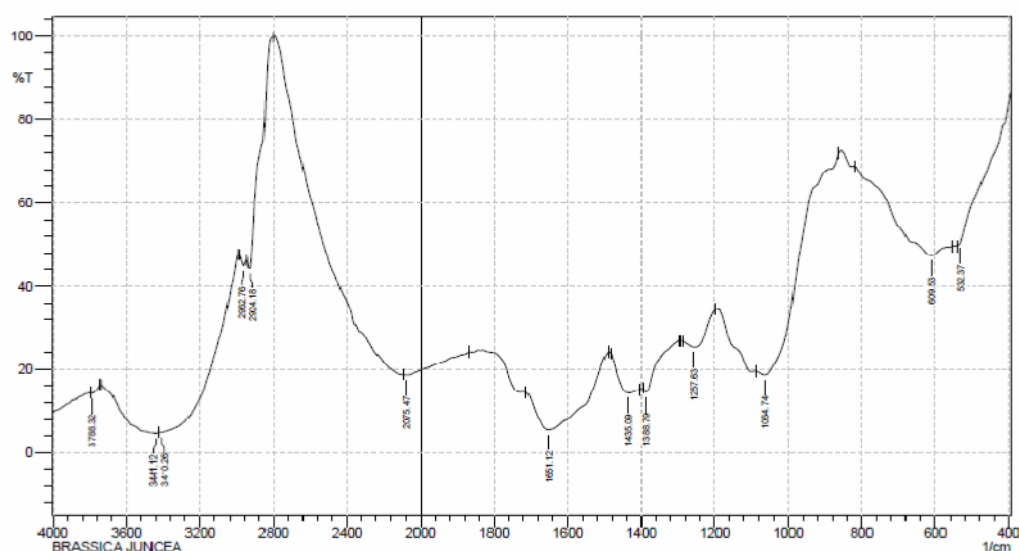
The methanolic extracts of the GLVs such as *B.juncea*, *T.foenum*, *A.polygonoides*, *A.tristis* and *S.grandiflora* were subjected to UV visible spectrophotometer at different wavelength from 250-800nm. The visible range of 400-700nm was taken into consideration. All the GLV extracts in common have peaks in between 650-700nm. The methanolic extract of *B.juncea* has another peak in between 400-450. All the other GLVs have an elevated peak at 400nm.

4.4.2 FT-IR analysis of the selected GLVs grown in different treatments and selected dyes in silk dyeing effluent

4.4.2.1 FT-IR spectrum of *B.juncea* grown in fresh water

Figure 26 shows the spectrum of dry powder of *B.juncea* grown in fresh water subjected to FT-IR (Fourier Transform Infrared Spectroscopy -IR affinity).

Figure 26
FT-IR spectrum of *B.juncea* grown in fresh water

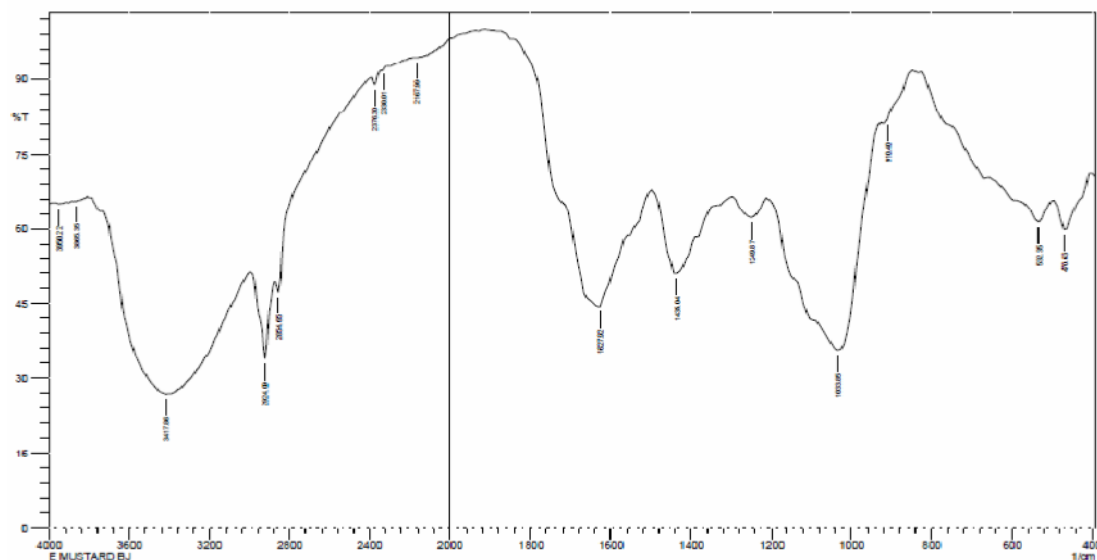


The FT-IR spectrum contain the following functional groups detected at 3441, 3410 wavenumber cm^{-1} has strong broad intensity which represents alcohol group with H-bonded stretch. The small sharp cluster of peaks at 2962, 2924 wavenumber cm^{-1} indicates the alkanes with C-H stretch. The 2075 wavenumber cm^{-1} corresponds to isocyanide ($\text{R}-\text{N}=\text{C}=\text{S}$). The medium peak of 1651 wavenumber cm^{-1} with $-\text{C}\equiv\text{C}-$ stretch was identified as alkenes. The wavenumber 1435 cm^{-1} has a C-C stretch of aromatic group and with variable bending in 1388 wavenumber cm^{-1} represents an alkane group. The 1257 wavenumber cm^{-1} with C-N stretch of small peak shows the presence of amine group presence. The C-O stretch of 1067 wavenumber cm^{-1} indicates alcohol group. The wavenumber 609 cm^{-1} has C-H bend corresponds to alkynes. The 532 wavenumber cm^{-1} with C-Br stretch belongs to alkyl halide.

4.4.2.2 FT-IR spectrum of *B.juncea* grown in crude effluent

Figure 27 illustrates the spectrum of dry powder of *B.juncea* grown in crude silk dyeing effluent subjected to FT-IR.

Figure 27
FT-IR spectrum of *B.juncea* grown in crude effluent



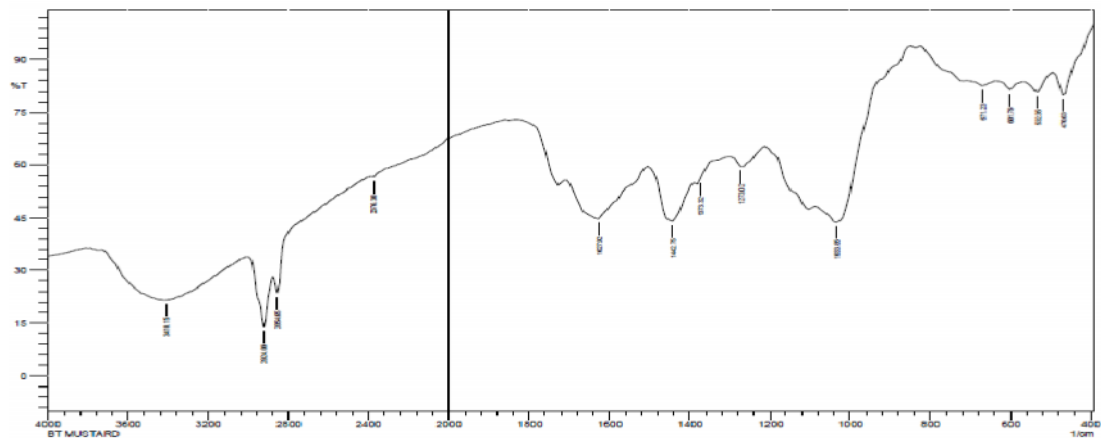
The FT-IR spectrum comprises 3417 wavenumber cm^{-1} with strong and broad peak indicates O-H bond of alcohol/phenols. Similar to the previous spectrum 2924, 2854 wavenumber cm^{-1} with -C-H- stretch corresponds to alkane. The 2376, 2330 wavenumber cm^{-1} did not match with the FT-IR standard chart and are not detectable. The 2167 wavenumber cm^{-1} with $-\text{C}\equiv\text{C}-$ stretch shows alkyne group. The C=C Stretch of conjugated double bond with 1627 wavenumber cm^{-1} correspond to alkenes. The characteristic absorbance bands at 1435, 910 and 532 wavenumber cm^{-1} represents aromatics (-C=C- stretch), primary/secondary amine, alkyl halide respectively. The 1249, 1033 wavenumber cm^{-1} corresponds to aliphatic amine (-C-N- Stretch).

4.4.2.3 FT-IR spectrum of *B.juncea* grown in biotreated effluent

Figure 28 reveals the spectrum of dry powder of *B.juncea* grown in biotreated effluent subjected to FT-IR.

Figure 28

FT-IR spectrum of *B.juncea* grown in biotreated effluent



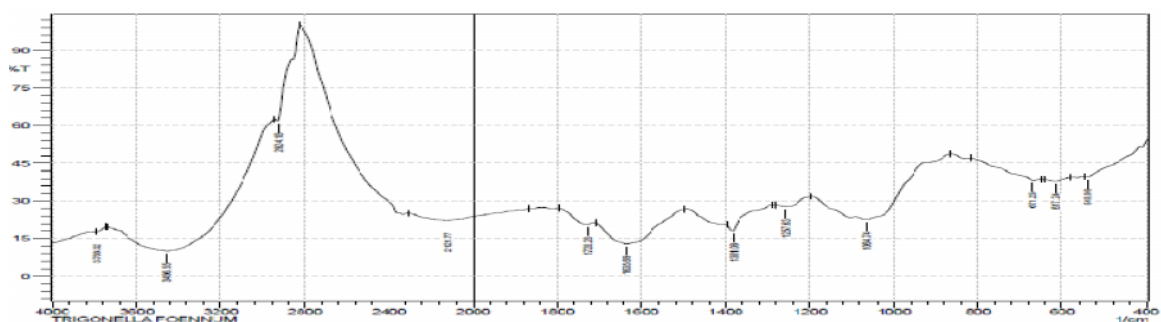
The spectrum with some peaks which has similarity in the wavenumber 3410, 2924, 1627, 532 cm^{-1} signifies alcohol/phenols (OH Stretch, H bonded), alkane (C-H- Stretch), alkene, alkyl halide similar to the spectrum of *B.juncea* grown in effluent. The other wavenumber cm^{-1} at 1442 belongs to aromatics (-C-C- Stretch). The small peaks at 671 and 601 wavenumber cm^{-1} corresponds to alkyl halide group with -C-Cl- stretch and -C-Br- stretch respectively.

4.4.2.4 FT-IR spectrum of *T.foenum* grown in fresh water

Figure 29 shows the spectrum of dry powder of *T.foenum* grown in fresh water subjected to FT-IR.

Figure 29

FT-IR spectrum of *T.foenum* grown in fresh water



The FT-IR spectrum of *T.foenum* with different characteristic absorption bands. The alcohol group has a strong and broad peak of intensity at 3456 and 1064 wavenumber cm^{-1} with O-H and C-O stretch respectively. The alkane group has -C-H- stretch at 2924

wavenumber cm^{-1} . The wavenumber 2121 cm^{-1} absorption band has a shallow intensity indicates alkyne. The $\text{C}=\text{O}$ stretch at wavenumber 1728 cm^{-1} with small, narrow band represents carboxylic acids. The alkene group was detected at 1635 and 1381 wavenumber cm^{-1} . The 1257 wavenumber cm^{-1} with $\text{C}-\text{N}$ stretch of small peak corresponds to amine group. The alkyl halide has its presence of $-\text{C}-\text{Cl}-$ stretch at $617, 671$ wavenumber cm^{-1} and $-\text{C}-\text{Br}-$ stretch at 570 wavenumber cm^{-1} .

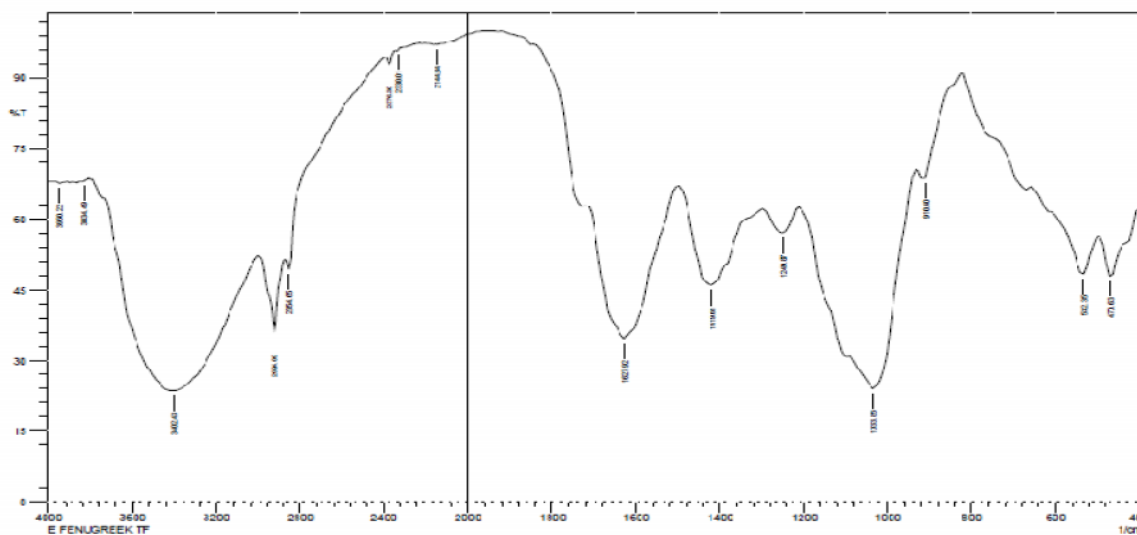
4.4.2.5 FT-IR spectrum of *T.foenum* grown in effluent

Figure 30 illustrates the spectrum of *T.foenum* grown in 75% of silk dyeing effluent subjected to FT-IR.

The FT-IR spectrum of *T.foenum* grown in effluent has a strong, broad band of intensity at 3402 wavenumber cm^{-1} which represents alcohol group. The alkane group has a absorption band at 2924 and 2854 wavenumber cm^{-1} . Also an amine group was found at various intensities at 1627 cm^{-1} , 1249 cm^{-1} and 910 cm^{-1} . The 2376 and 2330 wavenumber cm^{-1} was not detected.

Figure 30

FT-IR spectrum of *T.foenum* grown in effluent



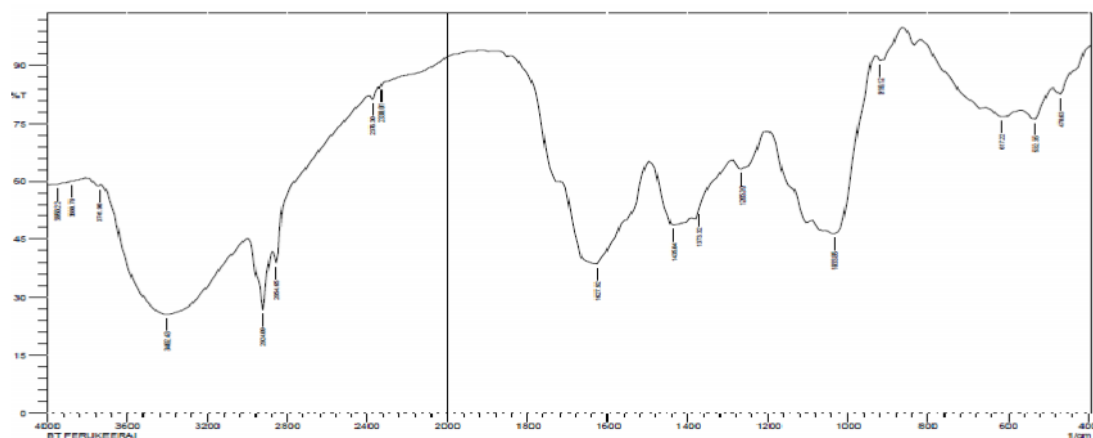
Note: *T.foenum* plant did not survive on the 45th day of 100% effluent, so 75% effluent affected plants were subjected to FTIR analysis

4.4.2.6 FT-IR spectrum of *T.foenum* grown in biotreated effluent

Figure 31 depicts the spectrum of dry powder of *T.foenum* grown in biotreated effluent subjected to FT-IR.

Figure 31

FT-IR spectrum of *T.foenum* grown in biotreated effluent



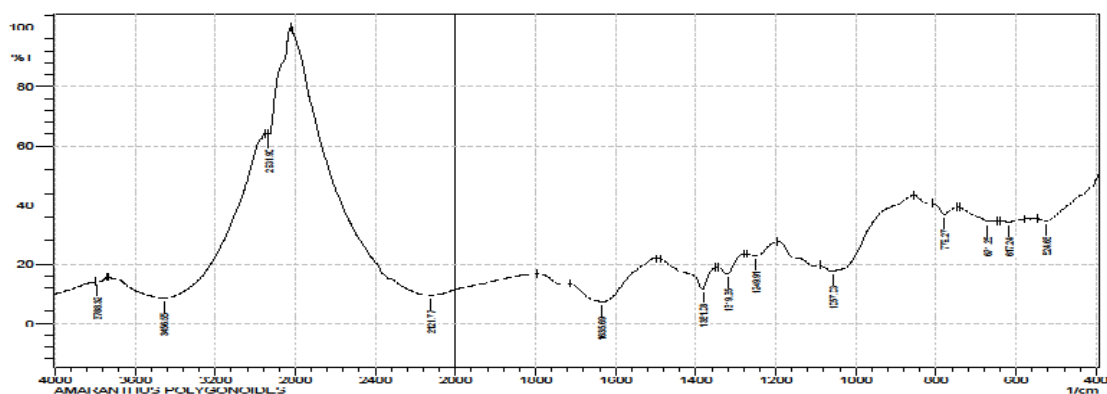
The FT-IR spectrum of characteristic absorption has the similar wavenumber 2924 cm^{-1} with similar functional group as in the spectrum obtained for the *T.foenum* grown in effluent. The alkyl halide has absorption band at 1265 , 617 and 532 wavenumber cm^{-1} of different intensity stretch. The new detected wavenumber 918 cm^{-1} with N-H Wag belongs to primary and secondary amine.

4.4.2.7 FT-IR spectrum of *A.polygonoides* grown in fresh water

The spectrum of dry powder of *A.polygonoides* grown in fresh water subjected to FT-IR was illustrated in Figure 32.

Figure 32

FT-IR spectrum of *A.polygonoides* grown in fresh water



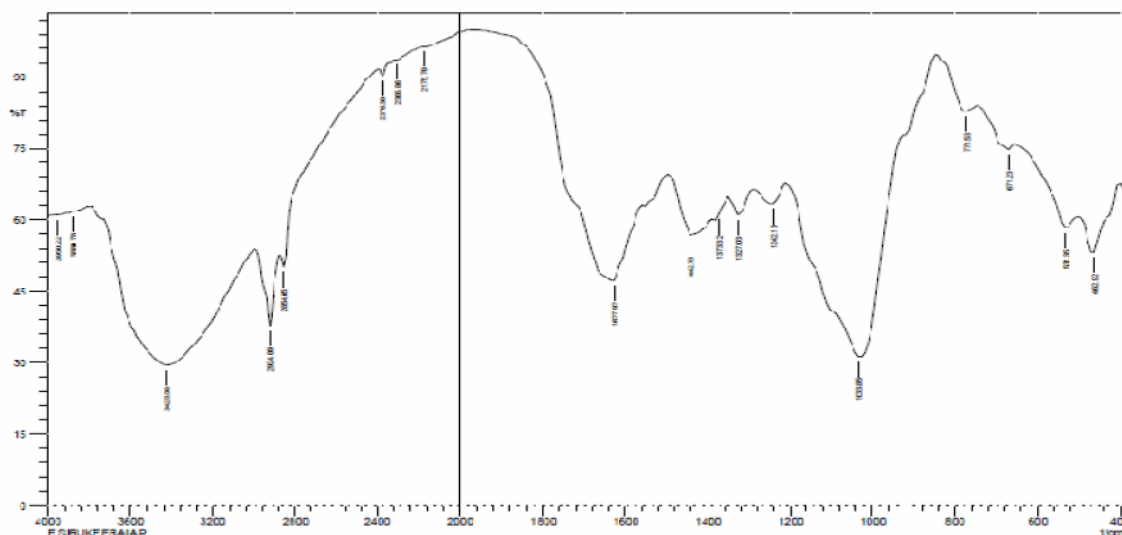
The FT-IR spectrum of *A. polygonoides* grown in fresh water has symbolized 12 absorption bands. The characteristic band at 3456 wavenumber cm^{-1} of strong broad intensity was an alcohol group and 2931 wavenumber cm^{-1} with C-H Stretch belongs to alkane group.

The alkyne group with C=C stretch indicated at 2121 wavenumber cm^{-1} peak. The variable intensity with N-H bend embody at 1635 cm^{-1} peak. The N-O symmetric stretch at 1381 and 1319 wavenumber cm^{-1} peak related to nitro groups. The 1249 and 1057 wavenumber cm^{-1} peak of absorbance with C-O stretch was resemblance to alcohols, carboxylic acids, esters and ethers. All the other peaks such as 779, 671, 617 and 524 wavenumber cm^{-1} with small peaks are due to alkyl halide.

4.4.2.8 FT-IR Spectrum of *A. polygonoides* grown in crude effluent

Figure 33 shows the spectrum of dry powder of *A. polygonoides* grown in crude silk dyeing effluent subjected to FT-IR.

Figure 33
FT-IR Spectrum of *A. polygonoides* grown in crude effluent



The FT-IR spectrum of *A. polygonoides* grown in crude silk dyeing effluent encompasses the already found absorption bands such as 3425 wavenumber cm^{-1} which correlates to alcohol/phenol. The other effluent exposed GLVs spectrum of some spectral bands like 1627, 1033 and 532 wavenumber cm^{-1} were found in this spectrum.

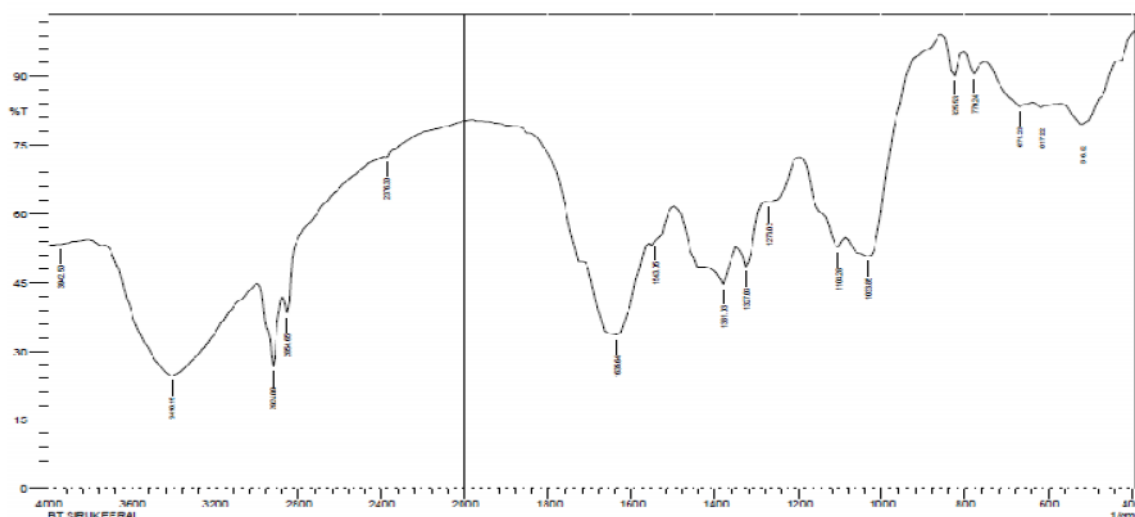
The P-H stretch of phosphines group at 2308 wavenumber cm^{-1} was found. The amine group with aromatic and aliphatic was seen at the bands 1327 and 1242 wavenumber cm^{-1} respectively.

4.4.2.9 FT-IR spectrum of *A. polygonoides* grown in biotreated effluent

Figure 34 depicts the spectrum of dry powder of *A. polygonoides* grown in biotreated effluent subjected to FT-IR.

Figure 34

FT-IR spectrum of *A. polygonoides* grown in biotreated effluent



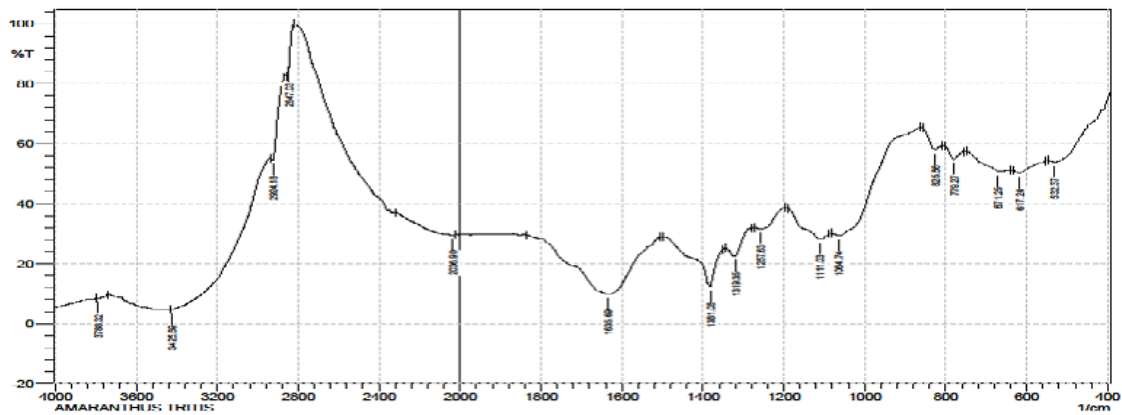
The FT-IR spectrum of biotreated *A. polygonoides* plant exemplify with $-\text{CH}$ stretch of alkane group. The amine groups detected in the spectral bands 1635, 1327 and 1033 wavenumber cm^{-1} corresponds to N-H bend and $-\text{C}-\text{N}$ stretch respectively. The wavenumber 1543 and 1381 wavenumber cm^{-1} with characteristic absorption bands reported to be nitrocompounds. The alkyl halide with $-\text{Cl}-$ stretch was depicted at 825 and 779 wavenumber cm^{-1} and $-\text{C}-\text{Br}-$ stretch at 516 wavenumber cm^{-1} . The 671 wavenumber cm^{-1} with $-\text{CH}-$ stretch indicates aromatics and 617 cm^{-1} with C-H bend stand up to alkyne group.

4.4.2.10 FT-IR spectrum of *A. tristis* grown in fresh water

Figure 35 illustrates the spectrum of dry powder of *A. tristis* grown in fresh water subjected to FT-IR.

Figure 35

FT-IR spectrum of *A. tristis* grown in fresh water



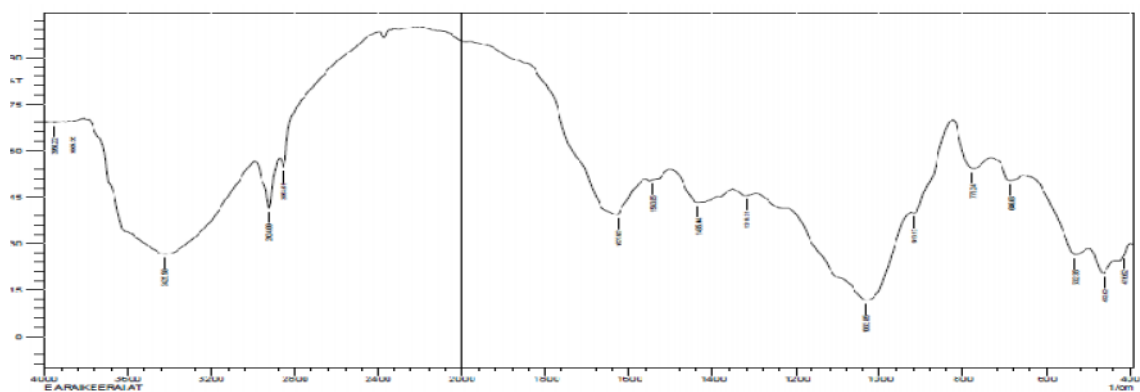
The FT-IR spectrum of *A. tristis* grown in fresh water has significant 12 absorption bands. The band of strong broad intensity at 3425 wavenumber cm^{-1} corresponds to alcohol group. The bands 2924 and 2847 wavenumber cm^{-1} with C-H stretch belongs to alkane group. The alkynes group with C=C stretch indicated at wavenumber 2121 cm^{-1} peak. The N-H bend symbolized at 1635 cm^{-1} peak reveals the amide group. The absorption bands at wavenumber 1381 and 1319 cm^{-1} peak with N-O symmetric stretch related to nitro groups. The wavenumber 1111 cm^{-1} peak of absorbance with C-O stretch has resemblance to alcohols, carboxylic acids, esters and ethers. All the other peaks such as 825, 779, 671, 617 and 524 wavenumber cm^{-1} with small peaks related to alkyl halide.

4.4.2.11 FT-IR spectrum of *A. tristis* grown in crude effluent

Figure 36 depicts the spectrum of *A. tristis* grown in crude silk dyeing effluent subjected to FT-IR.

Figure 36

FT-IR spectrum of *A. tristis* grown in crude effluent

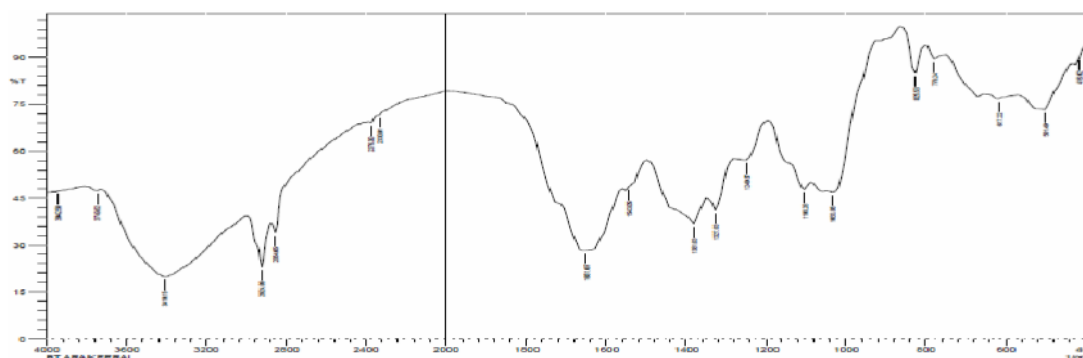


The FT-IR spectrum of *A.tristis* grown in crude effluent has some peaks at 3425 wavenumber cm^{-1} of alcohol group, 2924 and 2854 wavenumber cm^{-1} of alkane group, 1319 wavenumber cm^{-1} of alcohols, carboxylic acids, esters, ethers and 532 wavenumber cm^{-1} of alkyl halide. The aromatic group was found at the peak 1435 wavenumber cm^{-1} . Some spectral bands were viewed at 1627, 1033, 918 wavenumber cm^{-1} belong to primary amine, alcohols/ carboxylic acids/ esters/ether, primary and secondary amines respectively. The alkyl halide spectrum were noted at 779, 686 and 532 wavenumber cm^{-1} .

4.4.2.12 FT-IR spectrum of *A.tristis* in biotreated effluent

Figure 37 shows the spectrum of *A.tristis* grown in biotreated effluent subjected to FT-IR.

Figure 37
FT-IR spectrum of *A.tristis* in biotreated effluent

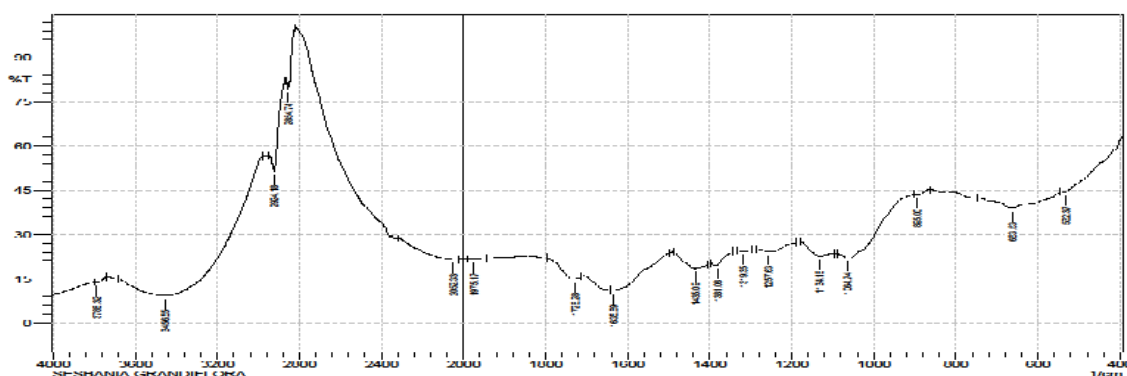


The FT-IR spectrum of *A.tristis* grown in biotreated water resembles to some important absorption bands of already discussed functional groups of wavenumber 3410, 2924 and 2854 cm^{-1} corresponds to alcohol and alkane groups. The indefinite spectral bands of 2376 and 2330 wavenumber cm^{-1} were also noted. The nitro groups of 1543 and 1381 wavenumber cm^{-1} correlates with N-O stretch. The amine group was identified at wavenumber 1327 and 1249 cm^{-1} . The bands of 1103 and 1033 wavenumber cm^{-1} , corresponds to alcohols/ carboxylic acids/ esters/ether group of different stretch of C-O and C-N respectively. The alkyl halide group was confirmed at the spectral bands of 779, 617 and 501 wavenumber cm^{-1} .

4.4.2.13 FT-IR spectrum of *S.grandiflora* grown in fresh water

Figure 38 reveals the spectrum of *S.grandiflora* grown in fresh water subjected to FT-IR.

Figure 38
FT-IR spectrum of *S.grandiflora* grown in fresh water

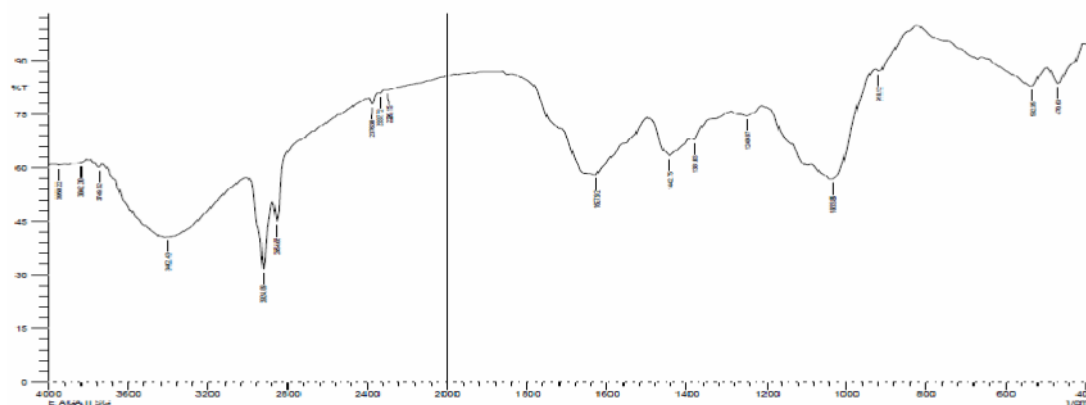


The FT-IR spectrum of *S.grandiflora* grown in fresh water has considerable absorption bands. The band of strong broad intensity at 3456 wavenumber cm^{-1} belongs to alcohol group and 2924 and 2847 wavenumber cm^{-1} with C-H stretch belongs to alkane group. The isocyanide group indicated at 2052 and 1975 wavenumber cm^{-1} peak. The N-H bend exemplifies at 1635 wavenumber cm^{-1} peak reveals the amide group. The absorption bands at 1381 and 1319 wavenumber cm^{-1} peak with N-O symmetric stretch correspond to nitro groups. The wavenumber 1134 and 1064 cm^{-1} peak of absorbance has resemblance to aliphatic amine and 1435, 895 wavenumber cm^{-1} correlates to aromatics groups. All the other peaks such as 1257, 663 and 532 wavenumber cm^{-1} with small bands corresponds to alkyl halide.

4.4.2.14 FT-IR spectrum of *S.grandiflora* grown in crude effluent

Figure 39 shows the spectrum of *S.grandiflora* grown in crude silk dyeing effluent subjected to FT-IR.

Figure 39
FT-IR spectrum of *S.grandiflora* grown in crude effluent



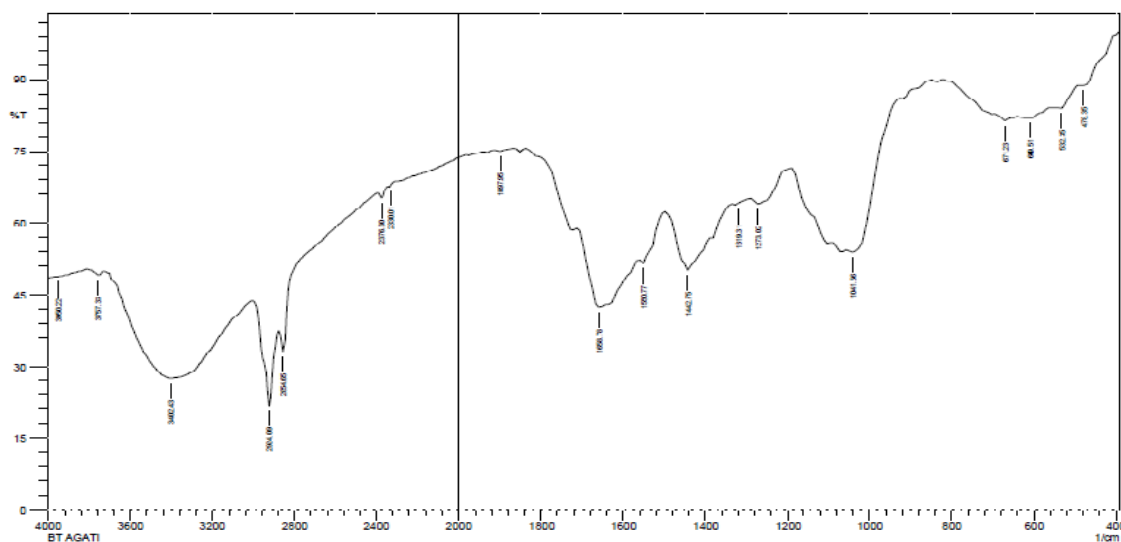
The FT-IR spectrum represents the characteristic absorption bands at 2924 and 2854 wavenumber cm^{-1} as previously discussed to be alkane and bands such as 2376 and 2337 wavenumber cm^{-1} were not detected. Also wavenumber 1381 and 532 cm^{-1} of spectral bands were similar to the *S.grandiflora* grown in fresh water. The wavenumber 1381, 1249 and 918 cm^{-1} confirms the amine groups of various categories.

4.4.2.15 FT-IR spectrum of *S.grandiflora* grown in biotreated effluent

Figure 40 represents the spectrum of *S.grandiflora* grown in biotreated effluent subjected to FT-IR.

Figure 40

FT-IR spectrum of *S.grandiflora* grown in biotreated effluent



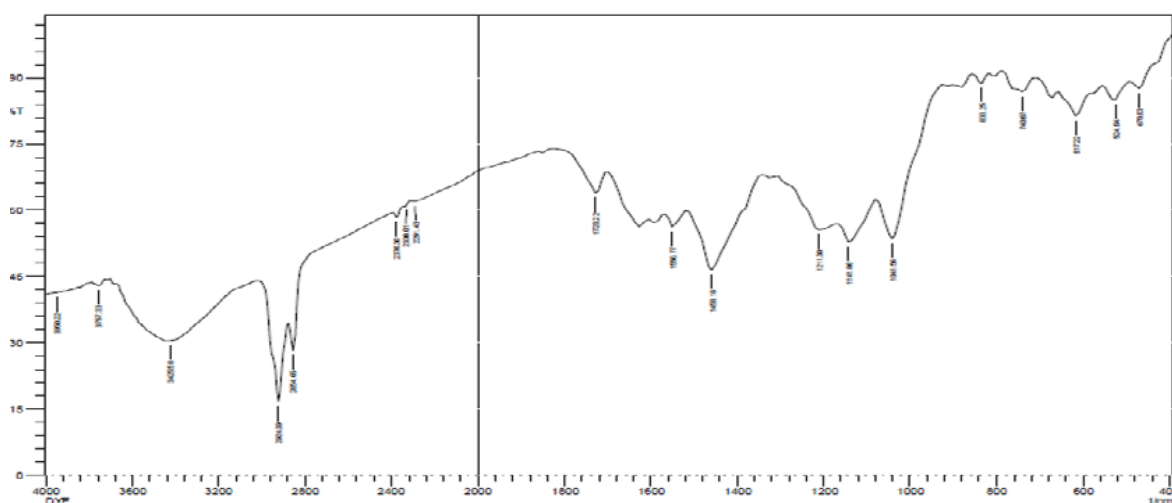
The FT-IR spectrum of *S.grandiflora* grown in biotreated effluent of spectral bands detected at 3402, 2924 and 2854 cm^{-1} indicates the alcohol and alkane respectively. These bands were repeated throughout the spectral study in other GLVs. The nitro groups were observed with the N-O stretch intensity at 1550 and 1319 cm^{-1} and amine group detected at 1273 and 1041 wavenumber cm^{-1} with C-N stretch. The alkyl halide groups were also revealed at few bands absorbed at wavenumber 671, 609 and 532 cm^{-1} .

4.4.2.16 FT-IR spectrum of the selected dyes of silk dyeing effluent

Figure 41 shows the spectrum of dry powder of selected dyes (Direct 2y2, Direct yellow 5gll, Procell pineapple) subjected to FT-IR.

Figure 41

FT-IR spectrum of the selected dyes of silk dyeing effluent



The dye powder subjected to FT-IR analysis confirms the presence of alcohol, alkane, alkynes, aldehyde, nitro groups, aliphatic amines, primary and secondary amine, aromatic group and alkyl halide groups.

4.4.2.17 Functional groups detection from FT-IR spectrum of selected GLVs

Table 8 depicts the analysis of functional groups in the selected GLVs of different treatments.

Thus from the functional group studies by FT-IR, the alcohol, alkane, alkyl halide and amine groups were found in all the GLVs irrespective of the treatments, even in crude effluent, the plants managed to synthesize these organic compounds. The amide group was completely absent in all the treatments. The phosphine group was detected only in *A.polygonoides* plant grown in crude effluent. The isocyanide group was found only in *B.juncea*, *A.polygonoides* and *S.grandiflora* grown in fresh water, which was unable to synthesize isocyanide group in plants grown in crude effluent and biotreated effluent. Irrespective of the treatments, the aromatic groups were synthesized by *B.juncea*, *A.tristis* and *S.grandiflora* as well as *T.foenum* grown in biotreated effluent and *A.polygonoides* grown in both crude effluent and biotreated effluent. The alkene group was detected in different treatments of *B.juncea*, *T.foenum* and *S.grandiflora* as well as *A.polygonoides* grown in fresh water and *A.tristis* grown in biotreated effluent.

Table 8

Functional groups detected in the FT-IR spectra of different GLVs in different treatments

Functional group	<i>B.juncea</i>			<i>T.foenum</i>			<i>A.polygonoides</i>			<i>A.tristis</i>			<i>S.grandiflora</i>		
	BJN	BJE	BJT	TFN	TFE	TFT	APN	APE	APT	ATN	ATE	ATT	SGN	SGE	SGT
Alcohol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alkane	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alkene	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+
Alkynes	-	+	-	+	+	-	+	-	+	-	-	-	-	-	-
Carboxylic acid	-	-	-	+	+	+	+	+	+	+	+	+	-	+	-
Esters	-	-	-	-	+	+	+	+	+	+	+	+	-	+	-
Ethers	-	-	-	-	+	+	+	+	+	+	+	+	-	+	-
Isocyanide	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-
Phosphine	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Aromatic	+	+	+	-	-	+	-	+	+	+	+	+	+	+	+
Nitro groups	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
Amide	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aldehyde	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Amine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alkyl halide	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ Presence - Absence

BJN: *Brassica juncea*, TFN: *Trigonella foenum*, APN: *Amaranthus polygonoides*, ATN: *Amaranthus tristis*, SGN: *Sesbania grandiflora* were grown in fresh water. BJE: *Brassica juncea*, APE: *Amaranthus polygonoides*, ATE: *Amaranthus tristis*, SGE: *Sesbania grandiflora* were grown in crude effluent. TFE: *Trigonella foenum* grown in 75% effluent (*T.foenum* plant did not survive on the 45th day of 100% effluent, so 75% effluent exposed plants were subjected to FTIR analysis). BJT: *Brassica juncea*, TFT: *Trigonella foenum*, APT: *Amaranthus polygonoides*, ATT: *Amaranthus tristis*, SGT: *Sesbania grandiflora* were grown in biotreated effluent.

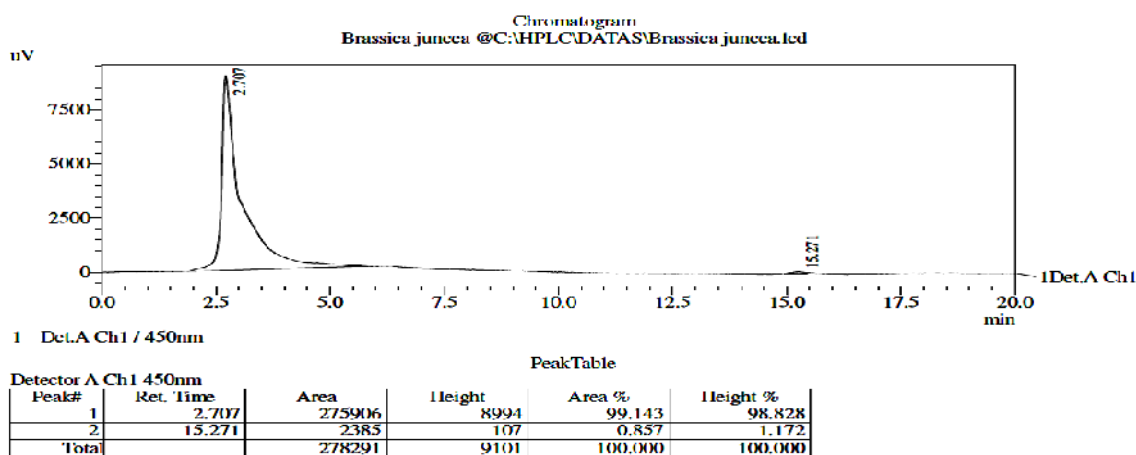
4.4.3. HPLC analysis of the methanolic extracts of the selected GLVs grown in different treatments, untreated and biotreated silk dyeing effluent

4.4.3.1 HPLC of *B.juncea* grown in fresh water

Figure 42 reveals the chromatogram of methanolic extract of *B.juncea* grown in fresh water which was subjected to High Pressure Liquid Chromatography (HPLC) analysis.

Figure 42

HPLC of *B.juncea* grown in fresh water

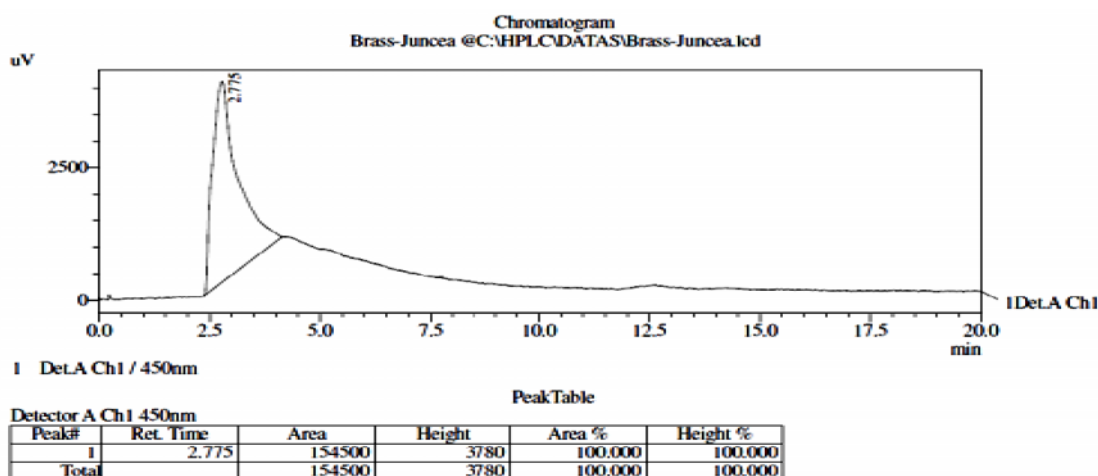


4.4.3.2 HPLC of *B.juncea* grown in crude effluent

Figure 43 depicts the chromatogram of methanolic extract of *B.juncea* grown in crude silk dyeing effluent.

Figure 43

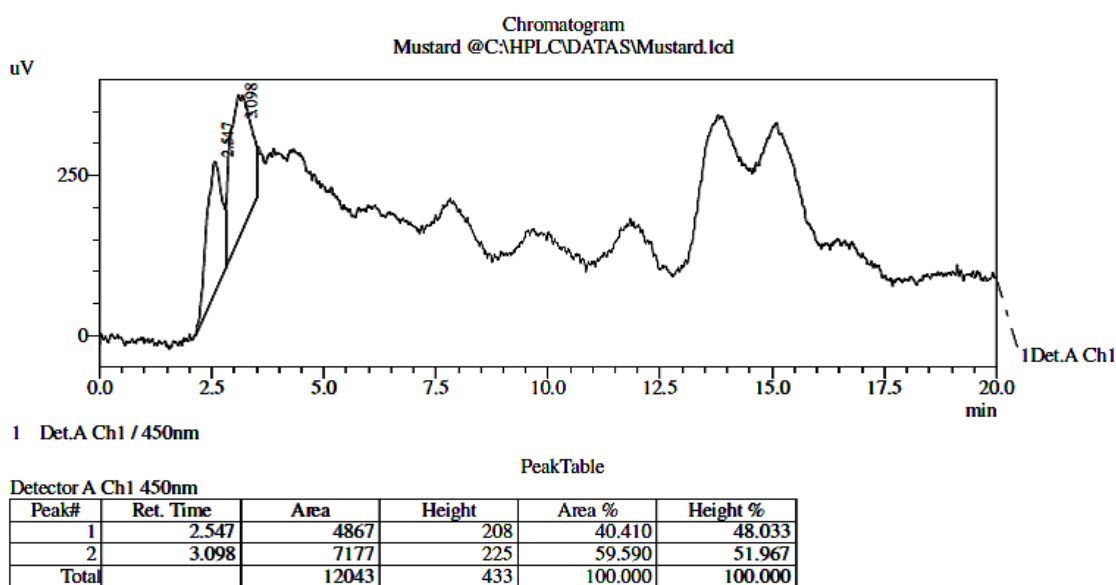
HPLC of *B.juncea* grown in crude effluent



4.4.3.3 HPLC of *B.juncea* grown in biotreated effluent

Figure 44 reveals the chromatogram of methanolic extract of *B.juncea* grown in biotreated effluent.

Figure 44
HPLC of *B.juncea* grown in biotreated effluent



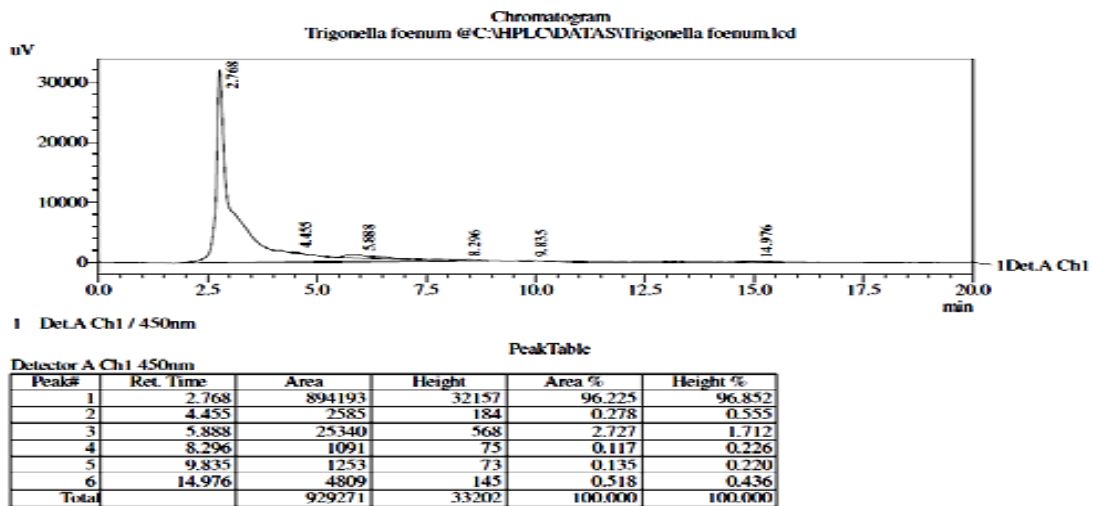
Figures 42, 43 and 44 shows the HPLC of different methanolic extracts of *B.juncea*. The *B.juncea* plant (BJN) grown in fresh water revealed about 2 peaks of retention time 2.7 and 15.2 minutes which had a broad and small peak respectively while the methanolic extracts of *B.juncea* (BJE) grown in crude effluent had shown one broad peak of retention time (tR) 2.7 minutes. The loss of a peak may be due to the effect of effluent that might have hindered the synthesis of the compound. While the biotreated *B.juncea* had shown two peaks of retention time (tR) 2.5 and 3.0 minutes respectively.

4.4.3.4 HPLC of *T.foenum* grown in fresh water

Figure 45 shows the chromatogram of methanolic extract of *T.foenum* grown in fresh water.

Figure 45

HPLC of *T.foenum* grown in fresh water

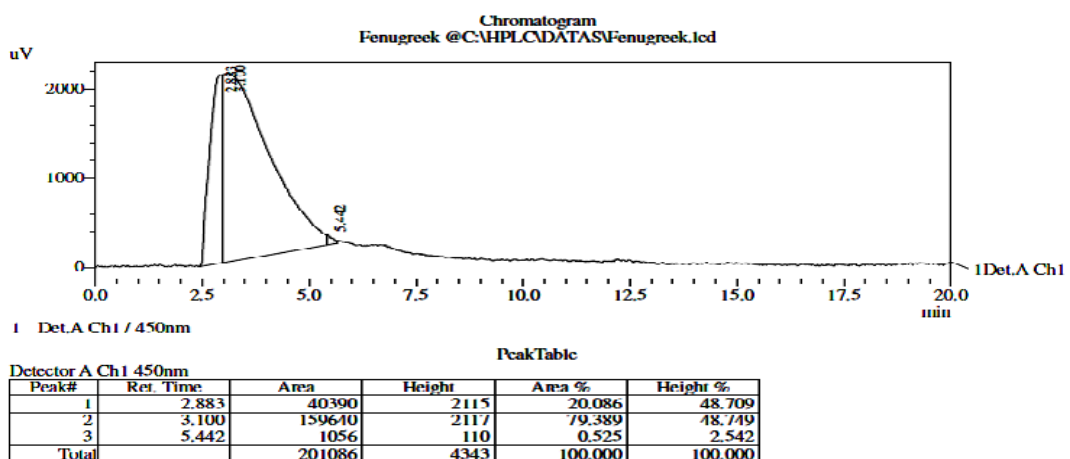


4.4.3.5 HPLC of *T.foenum* grown in effluent

Figure 46 illustrates the chromatogram of methanolic extract of *T.foenum* grown in 75% silk dyeing effluent.

Figure 46

HPLC of *T.foenum* grown in effluent

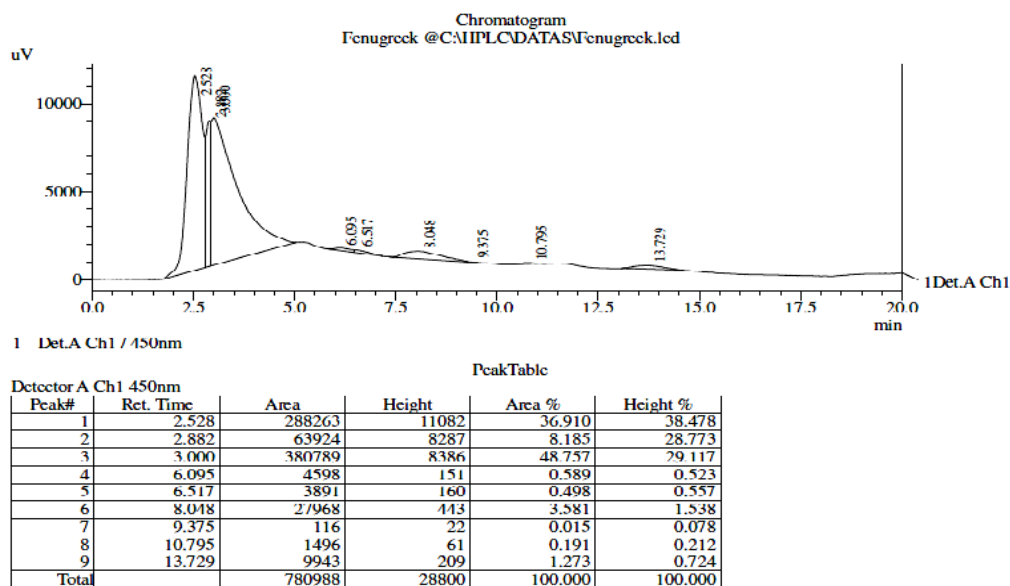


Note: *T.foenum* plant did not survive on the 45th day of 100% effluent, so 75% effluent exposed plants were subjected to HPLC analysis

4.4.3.6 HPLC of *T.foenum* grown in biotreated effluent

Figure 47 reveals the chromatogram of methanolic extract of *T.foenum* grown in biotreated effluent.

Figure 47
HPLC of *T.foenum* grown in biotreated effluent



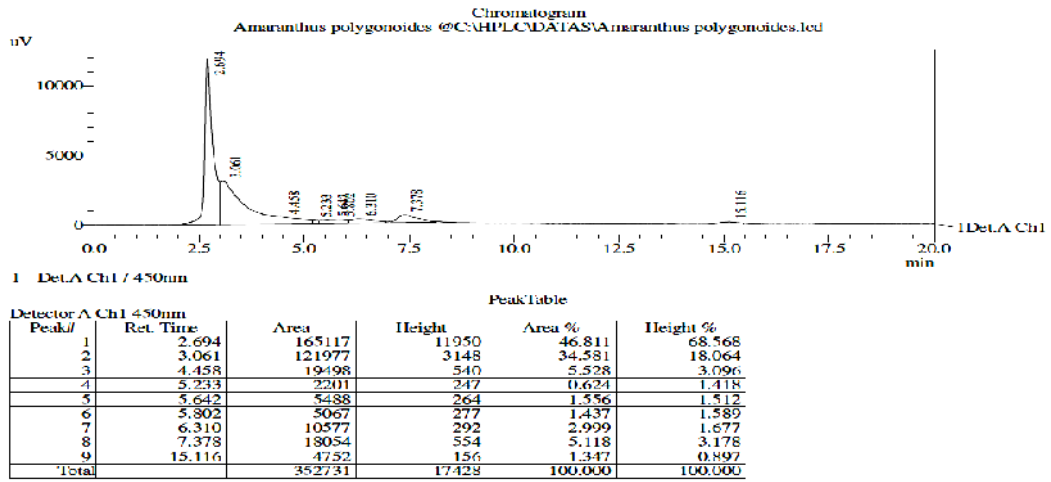
The HPLC analysis at wavelength of 450nm for the methanolic extract of *T.foenum* (TFN) grown in fresh water has 6 peaks of retention time (tR) 2.7, 4.4, 5.8, 8.2, 9.8 and 14.9 minutes on the other hand effluent exposed to plant (TFE) had shown only 3 peaks of tR 2.88, 3.1 and 5.4 minutes. The Chromatographic spectrum of biotreated plant has about 9 peaks of retention time (tR) 2.5, 2.8, 3.0, 6.0, 6.5, 8.0, 9.3, 10.7 and 13.7 minutes.

4.4.3.7 HPLC of *A.polygonoides* grown in fresh water

Figure 48 depicts the chromatogram of methanolic extract of *A.polygonoides* grown in fresh water. It was clear that the HPLC analysis of the methanolic extracts of *A.polygonoides* (APN) grown in fresh water has about 9 peaks of retention time (tR) of 2.6, 3, 4.4, 5.2, 5.6, 5.8, 6.3, 7.3 and 15.1 minutes. The broad peak at retention time (tR) of 2.6 occupies the largest % area of 46.8 and small peaks with retention time (tR) 3, 4.4, 5.2, 5.6, 5.8, 6.3, 7.3 and 15.11 minutes occupies the % area of 34.5, 5.5, 0.6, 1.5, 1.4, 2.9, 5.1 and 1.3 respectively.

Figure 48

HPLC of *A. polygonoides* grown in fresh water

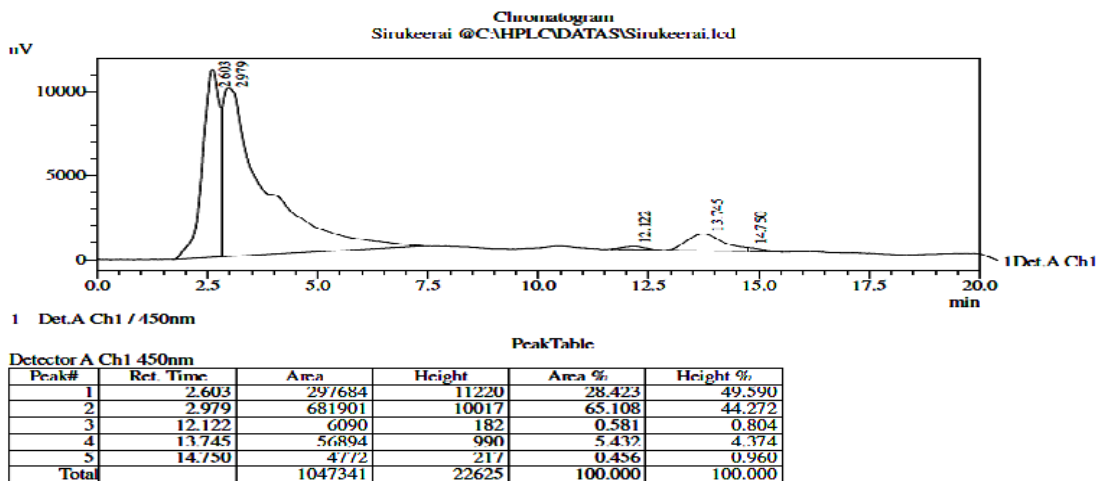


4.4.3.8 HPLC of *A. polygonoides* grown in crude effluent

Figure 49 illustrates the chromatogram of methanolic extract of *A. polygonoides* grown in crude effluent.

Figure 49

HPLC of *A. polygonoides* grown in crude effluent

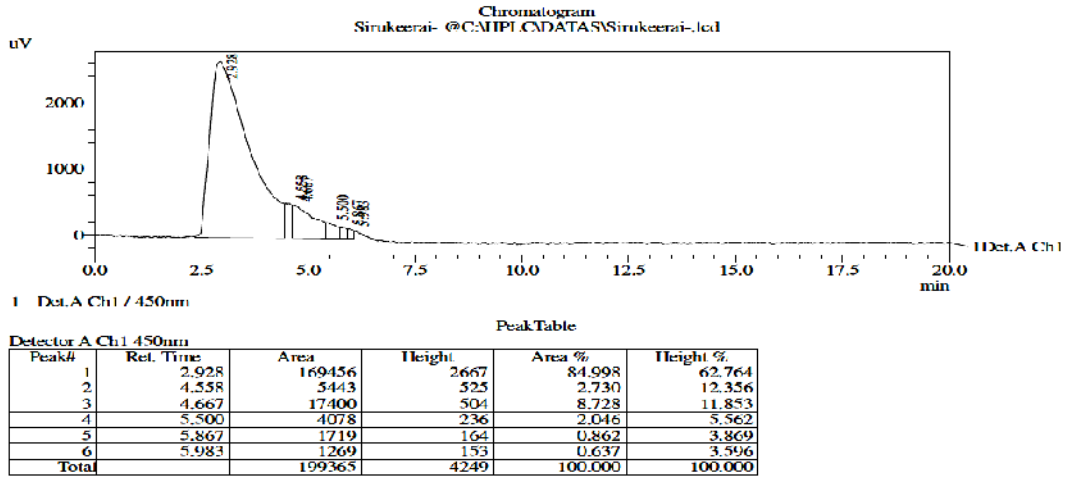


4.4.3.9 HPLC of *A. polygonoides* grown in biotreated effluent

Figure 50 reveals the chromatogram of methanolic extract of *A. polygonoides* grown in biotreated effluent.

Figure 50

HPLC of *A. polygonoides* grown in biotreated effluent



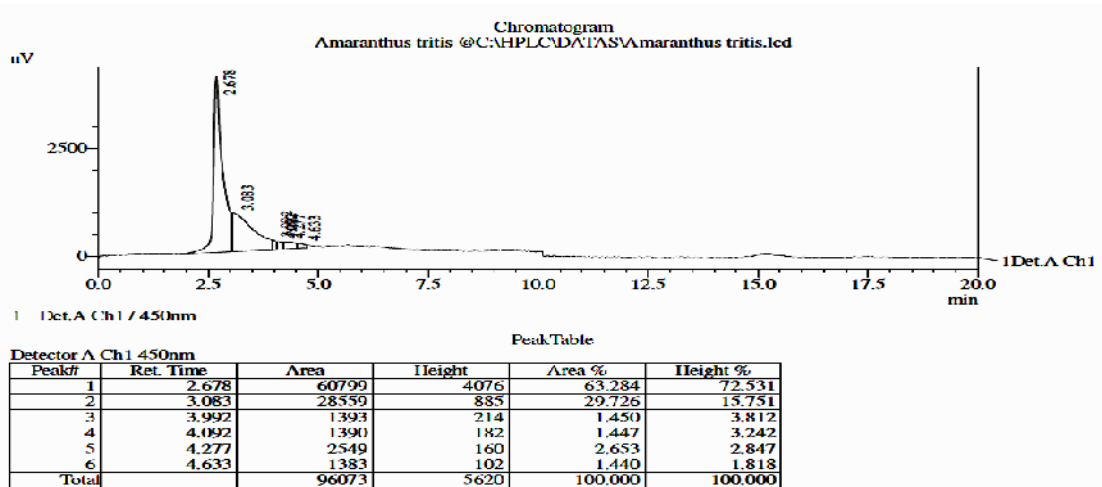
Whereas the methanolic extracts of the effluent exposed to *A. polygonoides* had shown 5 peaks but 6 peaks were found for biotreated *A. polygonoides* plant with the retention time of 2.9, 4.5, 4.6, 5.5, 5.8 and 5.9 minutes of maximum percentage area of 84.9.

4.4.3.10 HPLC of *A. tristis* grown in fresh water

Figure 51 explains the chromatogram of methanolic extract of *A. tristis* grown in fresh water.

Figure 51

HPLC of *A. tristis* grown in fresh water

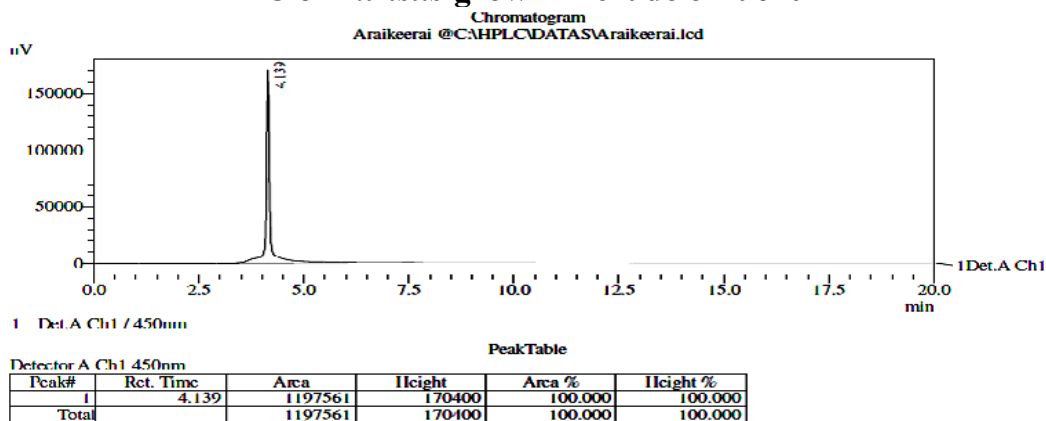


4.4.3.11 HPLC of *A.tristis* grown in crude effluent

Figure 52 illustrates the chromatogram of methanolic extract of *A.tristis* grown in crude silk effluent.

Figure 52

HPLC of *A.tristis* grown in crude effluent

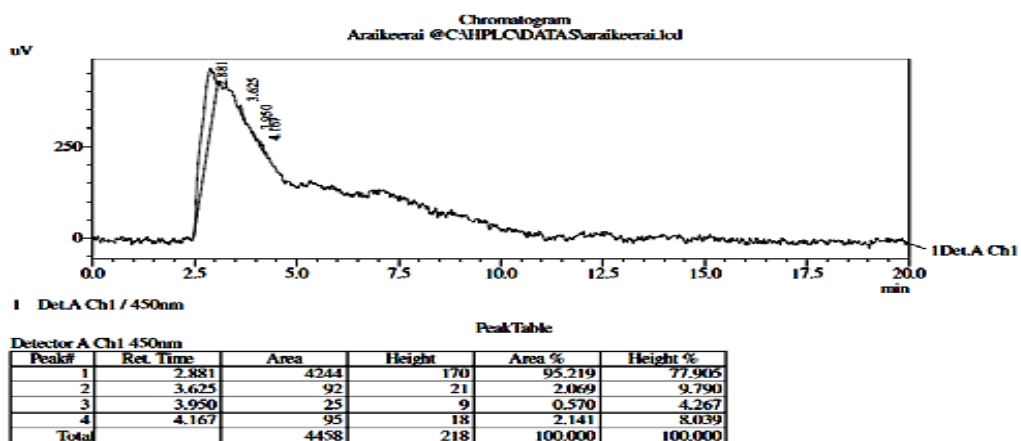


4.4.3.12 HPLC of *A.tristis* grown in biotreated effluent

Figure 53 reveals the chromatogram of methanolic extract of *A.tristis* grown in biotreated effluent.

Figure 53

HPLC of *A.tristis* grown in biotreated effluent

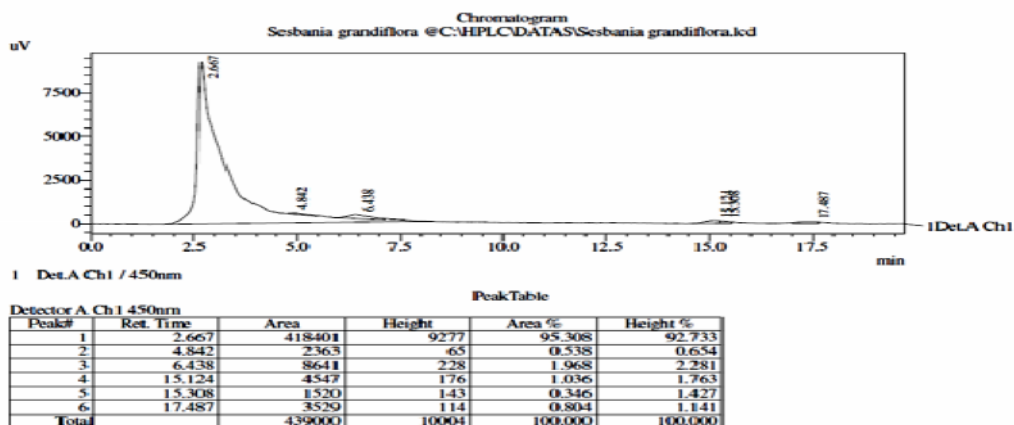


The HPLC results of methanolic extracts of *A.tristis* grown in fresh water has 6 peaks of retention time of 2.6, 3, 3.9, 4, 4.2, and 4.6 minutes whereas the effluent exposed plant had only one peak of retention time of 4.1 minutes. In the biotreated plant about 4 peaks were found with the maximum percentage area of 95.2%.

4.4.3.13 HPLC of *S.grandiflora* grown in fresh water

Figure 54 depicts the chromatogram of methanolic extract of *S.grandiflora* grown in fresh water.

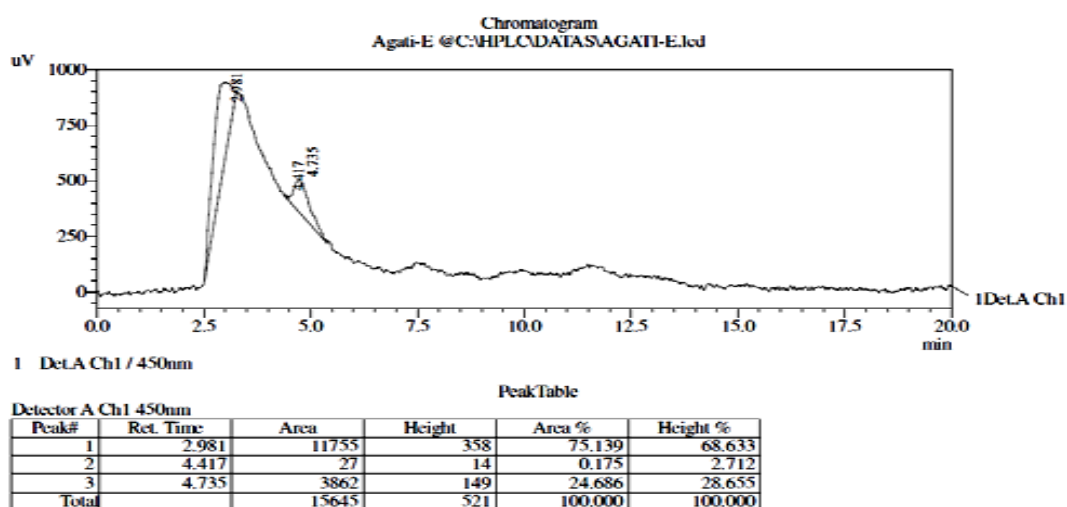
Figure 54
HPLC of *S.grandiflora* grown in fresh water



4.4.3.14 HPLC of *S.grandiflora* grown in crude effluent

Figure 55 views the chromatogram of methanolic extract of *S.grandiflora* grown in crude silk dyeing effluent.

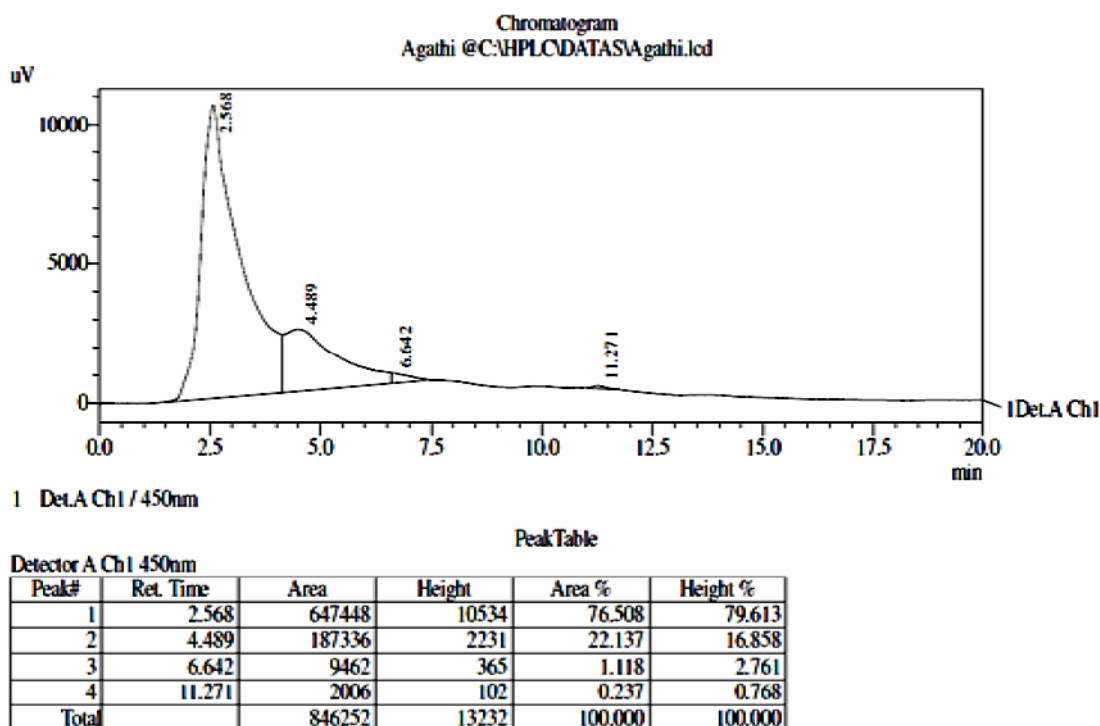
Figure 55
HPLC of *S.grandiflora* grown in crude effluent



4.4.3.15 HPLC of *S.grandiflora* grown in biotreated effluent

Figure 56 reveals the chromatogram of methanolic extract of *S.grandiflora* grown in biotreated effluent.

Figure 56
HPLC of *S.grandiflora* grown in biotreated effluent



The HPLC analysis of *S.grandiflora* grown in fresh water revealed 6 peaks of retention time 2.6, 4.8, 6.4, 15.1, 15.3 and 17.4 minutes of which only 3 peaks were present in *S.grandiflora* grown in effluent water. The smaller peaks of least percentage area 1.036, 0.346, 0.804 of retention time 15.1, 5.3, 17.4 minutes has been vanished from the chromatogram of effluent exposed plants. In the biotreated plant 4 peaks with the retention time of 2.5, 4.4, 6.6 and 11.2 minutes were found.

4.4.3.16 Standard HPLC of pigments, alkaloid and monosaccharides

Figure 57 shows the chromatogram of the standard pigments such as (Chl C₃, Chl C₂, Chl B and carotene), alkaloid (caffeine) and monosaccharides (glucose, fructose, mannose and galactose).

Figure 57

Standard HPLC of pigments, alkaloid and monosaccharides

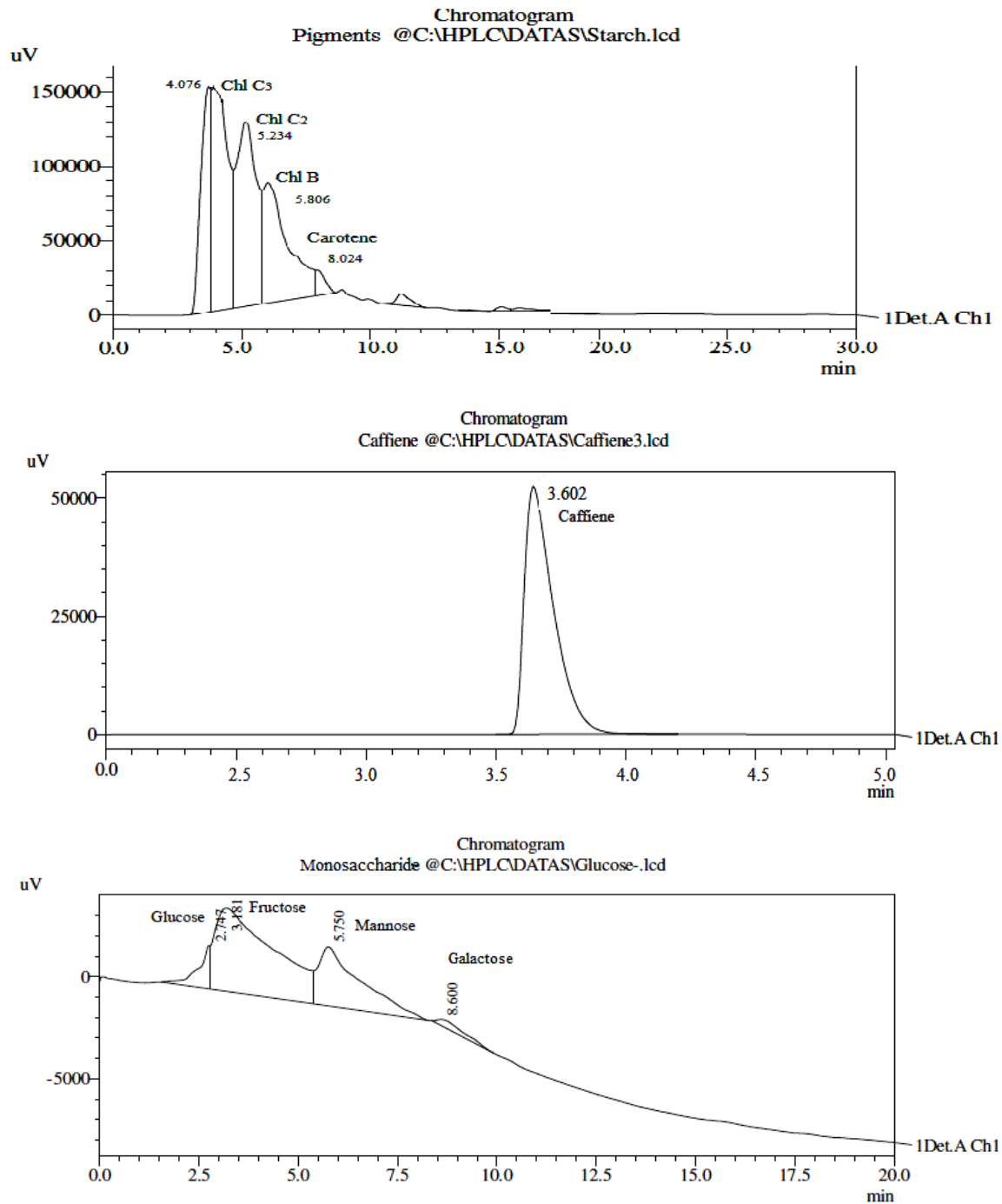


Figure 57 depicts the standard such as pigments of Chl C₃, Chl C₂, Chl B, carotene, alkaloid caffeine and monosaccharides such as glucose, fructose, mannose and galactose had peaks with the retention time (tR) of 4.0, 5.2, 5.8, 8.0, 3.6, 2.74, 3.18, 5.7 and 8.6 minutes respectively.

Table 9
Compounds detected in the HPLC of different GLVs in different treatments

Standards	Presence
Pigments	
Chlorophyll C ₃	ATN, ATE, ATT
Chlorophyll C ₂	APN
Chlorophyll B	TFN
Carotene	TFN, TFT
Alkaloid	
Caffeine	ATT
Monosaccharides	
Glucose	BJN, BJE, BJT, TFN, TFE, TFT, APN, APE, APT, ATN, ATE, ATT, SGN, SGE, SGT
Fructose	APN, TFE, TFT
Mannose	TFN
Galactose	TFN

BJN: *Brassica juncea*, TFN: *Trigonella foenum*, APN: *Amaranthus polygonoides*, ATN: *Amaranthus tristis*, SGN: *Sesbania grandiflora* were grown in fresh water.

BJE: *Brassica juncea*, APE: *Amaranthus polygonoides*, ATE: *Amaranthus tristis*, SGE: *Sesbania grandiflora* were grown in crude effluent. TFE: *Trigonella foenum* grown in 75% effluent (*T.foenum* plant did not survive on the 45th day of 100% effluent, so 75% effluent exposed plants were subjected to HPLC analysis)

BJT: *Brassica juncea*, TFT: *Trigonella foenum*, APT: *Amaranthus polygonoides*, ATT: *Amaranthus tristis*, SGT: *Sesbania grandiflora* were grown in biotreated effluent.

Thus from Table 9 and the chromatographic studies, the compounds were identified on comparison of these standards with the HPLC of methanolic extract of the selected GLVs of different treatments with the difference of ± 0.5 retention time (tR). Comparison of the retention time (tR) has revealed the presence of the pigments chlorophyll C₃ in ATN, ATE, ATT (*Amaranthus tristis* of normal, effluent and biotreated treatments), chlorophyll C₂ in APN (*Amaranthus polygonoides* grown in fresh water), chlorophyll B in TFN (*Trigonella foenum* grown in fresh water), carotene in TFN, TFT (*Trigonella foenum* grown in fresh water and biotreated effluent). The alkaloid caffeine

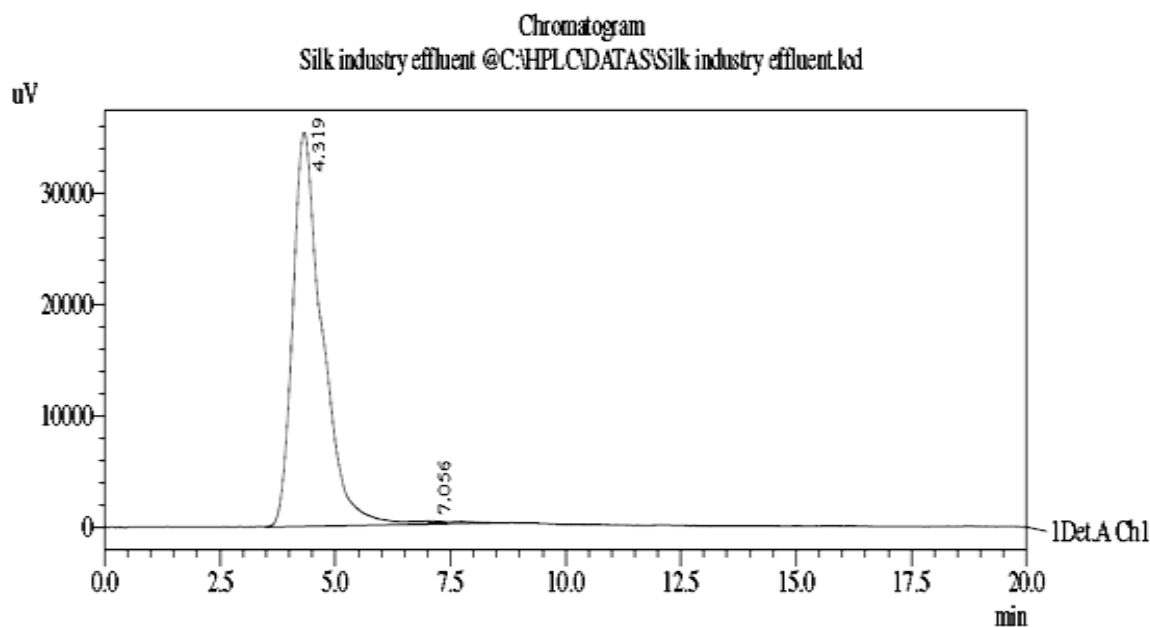
was found in ATT (*Amaranthus tristis* grown in biotreated effluent). The monosaccharide glucose was present in all the GLVs of all the treatments and fructose present in APN (*Amaranthus polygonoides* in fresh water), TFE and TFT (*Trigonella foenum* grown in crude effluent and biotreated effluent). The mannose and galactose were present only in TFN (*Trigonella foenum* grown in fresh water).

4.4.3.17 HPLC of silk dyeing industrial effluent

Figure 58 indicates the chromatogram of silk dyeing effluent.

Figure 58

HPLC of silk dyeing industrial effluent



1 Det.A Ch1 / 510nm

PeakTable

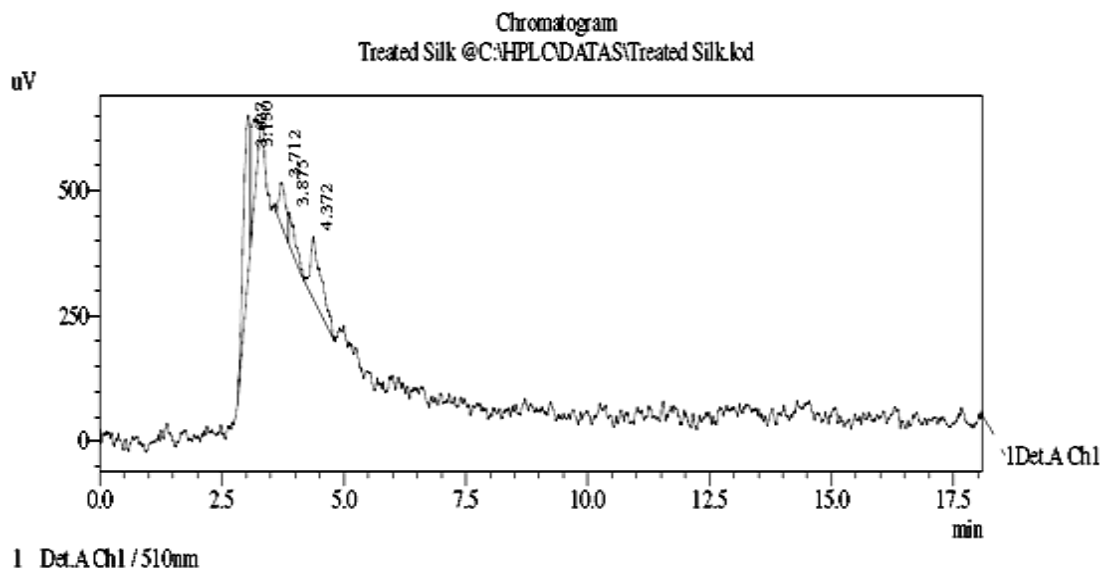
Detector A Ch1 510nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.319	1584582	35373	99.885	99.678
2	7.056	1827	114	0.115	0.322
Total		1586409	35488	100.000	100.000

4.4.3.18 HPLC of biotreated effluent

Figure 59 depicts the chromatogram of biotreated effluent by *Pseudomonas fluorescens*.

Figure 59
HPLC of biotreated effluent



PeakTable

Detector A Ch1 510nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.037	3271	319	39.010	41.232
2	3.150	1559	178	18.596	23.013
3	3.712	850	84	10.141	10.838
4	3.875	677	65	8.070	8.410
5	4.372	2028	128	24.182	16.507
Total		8386	773	100.000	100.000

Thus from the present study, the HPLC chromatogram of silk dyeing industrial effluent at the wavelength of 510nm has shown two peaks of retention time (tR) 4.3, 7.0 minutes with 99.88 % and 0.115 % area. The chromatogram of biotreated effluent has shown five peaks with retention time (tR) 3.0, 3.1, 3.7, 3.8 and 4.3 minutes with reduced percentage area of 39%, 18.5%, 10.1%, 8% and 24.18% which clearly indicates that the dye in the effluent has been degraded by *Pseudomonas fluorescens*.

Hence it can be concluded that the null hypothesis (H_0) “*Pseudomonas fluorescens* donot degrade the silk dyeing effluent” is rejected.